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INTRODUCTION

INTRODUCTION

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1. Sex Dimorphism in Physiology.

A wealth of data, clinical and experimental, points decisively to the conditioning action of sex hormones in a number of stressful situations. Although no generalization is possible in favor of one sex in many infectious, degenerative or metabolic conditions, sexual dimorphism proves a true variable in the survival of organisms under acute or chronic stress.

Females subjected to burns after fasting, survive better and longer than males (1), presumably because of protection resulting from a differential processing of the sex hormones by the liver (2). Differences in mortality appear due to the sex factor in a number of situations where toxicity reaches a lethal level (excess epinephrine, potassium, ethionine, picrotoxin)(3,4) or when oxygen becomes deficient (5); sex seems non-significant in burns applied to the fed anesthetized animal (6) and in anaphylactic shock in guinea pigs (7).

Radiation injury and lethality are modified by sex and sex hormones; testosterone propionate administered after X-irradiation, increases lethality of an LD₅₀ dose, while stilbestrol and estradiol, administered prior to irradiation, improve resistance in mice of both sexes (8, 9). Irradiation of the exteriorized gonads results in sterilization and cessation of endocrine activity at lower doses in the female than in the male; in the male, a notably higher resistance of the interstitium offers the possibility of "radiological dissection" of the testis into exocrine and endocrine components (10). Ovariectomy

increases the resistance to gamma radiation to the level of the male (11).

A dimorphic response to unspecific stress is seen in the serum cholesterol of monkeys (12), but the assumed protection by estrogens admits species differences (13). The well known sexual difference gives the heavier adrenal to the female rat and to the male hamster. Gonadectomy equalizes the situation by increasing the weight of the adrenal; androgens, but not estrogens, reverse the effect of gonadectomy. In the adrenalectomized hamster, the female, but not the castrate, survives longer and only progesterone (qualified by age) improves survival in both sexes; in the rat, progesterone and gonadotropins prove beneficial (14, 15).

The adrenal response to stress (undefined) represented by the level of plasma corticosterone, varies with age and sex, and is modified by gonadectomy in both sexes (16); the sex dimorphism carries even into the behaviour of cultured adrenals (17). Lathyrism caused experimentally by poisoning with amino-aceto-nitrile, is in part counterbalanced by casein, but the protection is lower in the female and in the male castrate (18).

The convulsive threshold to electroschock, lower in females, is depressed in males by estradiol (19). Liver damage caused by a single injection of CCl_4 proves more severe in the female; pre-treatment of the female with testosterone propionate attenuates the damage, reduces susceptibility and stimulates regeneration (20). A stress response with an undertone of sexual dimorphism to long-term

isolation was recently reported (21). A sexual difference appears in the survival of mature animals exposed to cold (2°C); the intact females and the male castrates survive longer, but ovariectomy causes no improvement. Exogenous hormones, estradiol or testosterone, prove detrimental (22).

Even in the absence of an alarm situation, several body functions display sexual variations.

The functional dimorphism of the adrenal parallels the morphological dimorphism under normal conditions (23, 24). In the rat and mouse, the female adrenal produces more steroids (absolutely and per unit mass of tissue), but testosterone depresses this activity to the male level (25, 26). The absolute amount of total lipids, of cholesterol and of phospholipids is greater in the female adrenal; even at a lower body weight, the female adrenal is notably heavier (27). The observed sexual difference (plasma corticosterone) is ascribed not to a difference in the rate of clearance or in the metabolism of the corticosteroids, but to a greater sensitivity of the female adrenal to ACTH stimulation and possibly to a greater responsiveness of the female pituitary to the stimulation of stress (28).

Some authors invoke a sexual difference in the behaviour of the pituitary-adrenal system for the interpretation of various experimental situations created by exogenous or endogenous sex hormones (29, 30, 31); even the growth process in the rat is differentially affected by exogenous testosterone and by gonadectomy (32, 33).

Unexplained sex differences have been detected in the function of the C.N.S. (34); among other observations, certain abnormalities in the reproduction pattern in some species are associated with electrical lesions in the pyriform cortex and amygdaloid complex (35).

Reasonable proof is offered of the existence of a "male component" in the liver proteins which would be the reason of a one-way histoincompatibility of skin homotransplants (based on the Y chromosome) from male to female (36); these immunological studies extended to amphibia have confirmed the cytogenetic basis in incompatibility genes (37). Two-dimensional electrophoresis gives a typical sex-associated pattern for alpha-1 and alpha-2 globulins which permits recognition of male and female serums (38). Mitochondria from intact female rats and mice oxydize added cholesterol to a greater extent than mitochondria from intact males (no difference in the oxydation of sodium propionate); castration of the male enhanced, and androgen to the female slightly depressed the process, but ovariectomy was without effect. Oxydation of cholesterol by preparations of normal male and female rat liver mitochondria was inhibited by sex hormones in vitro (39). Lipid mobilisation to the liver of the fasting animal occurs to a greater extent in the female than in the male (40).

In the rat, the metabolism of histamine is modified by the sex status: the female excretes much larger amounts of free histamine

than the male. Castration increases the free histamine output in the male, but testosterone reverses this effect; ovariectomy is without action. The male castrate and the female excrete greater amounts of exogenous histamine in free form (41). The difference is seen in the presence of a minor metabolic pathway (absent in the female) rather than in the biosynthesis (42).

Sex differences are seen in the sensitivity to estradiol (43), in the catabolism of tagged androgens (44) and in the pattern of radio-Iodine excretion in the urine of stilbestrol-treated guinea pigs (45). The current interest in the metabolism of cholesterol has led investigators into the study of the influence of sex and of age on the onset and development of atherosclerosis (46, 47, 48). The sexual difference in serum iron values in man has been long recognized; the few mammalian species examined have not shown uniformity in the effect of sex, gonadectomy and sex hormones (49, 50, 51). Significant differences detected in the ECG of "clinically healthy" humans (age 40-59) are attributed to sex; in consequence, caution is invited in the application to females of the present normal standards mainly derived from males (52).

The conditioning action of sex hormones (endogenous or exogenous) on the adaptive responses and on the normal body function seems to be an accepted fact of experimental and clinical physiology.

2. The case of the male organism.

The problem of age in the male and its genestic consequences, perennially baffles clinicians and investigators, as well as the eventual victims themselves.

Differences in glycogenesis and glycolysis in response to moderate cold in rats of various ages, lead to the assumption that the pattern of adaptation to stress may vary with age (53); age also modifies the response of the testis-accessory system, to exogenous testosterone (54). A recent report describes a form of lactic dehydrogenase present in the adult human testis, but absent in the prepubertal organ (55, 56). One should note that these observations emphasize a difference between adults and prepubertal subjects, but do not reveal changes within the period of sexual maturity.

Histochemical studies in the rooster show a progressive depression of several functional indices as age advances (57). Behavioral studies in rats point to the existence of an optimum region in the curve of sexual performance in terms of age (58). The male climacteric in the human remains a mystery: no abrupt changes are observed, although testicular androgens may be declining; replacement therapy has never produced the dramatic rejuvenation claimed or expected (59).

Various forms of radiant energy are known to affect adversely the testis. Abdominal temperatures, higher than scrotal temperatures,

are held responsible for the drastic germinal degeneration of the cryptorchid testis in all animal groups where the testis is normally or seasonally scrotal. In vitro studies of rabbit testis exposed to abdominal temperature for short periods (2, 6 hours), show an initial increase in metabolic activity followed by a significant decrease relative to the control; an exposition of 24 hours is not deleterious to the enzyme systems, but the decrease in glucose uptake corresponds with a decrease of available glucose resulting from the depressed blood flow and increased metabolic needs. The suggestion is made that spermatogenic arrest is caused by substrate deficiency (60, 61, 62).

Microwave irradiation (local or general) causes depression of the androgen output; the dorsolateral prostate fails to concentrate Zinc⁶⁵, but responds to chorionic gonadotropins and/or testosterone, indicating a decrease in LH output. The extent and duration of the damage are directly proportional to the dose and length of exposure, which may lead to third-degree burns and irreversible destruction of the interstitium (63, 64, 65, 66).

Considerable attention has been given to the effect of X-Rays on the male genital apparatus and function. X-Ray lethality is aggravated by sexual activity in mice, not only because of greater physical exertion but because of increased radio-sensitivity due to the presence of testosterone (67). Pretreatment with low doses reduces considerably the sensitivity to a single high dose (68).

The pattern of spermatogenic recovery in the boar (sperm number in periodical collections) is linearly (negative slope) related to the logarithm of the dose (69). Fractionated doses did not produce a different effect from that of a single equal dose (70). The nature of the damage to the germinal sequence in the human may be represented as a hastening of senescent changes: narrowing of the tubules, thickening of the tunica, progressive tubular fibrosis, increase in alkaline phosphatase, in glycogen and in phospholipids, but no change in acid phosphatase (71). The suggestion is made that total irradiation causes disruption in the pituitary-testes-prostate chain, whereby androgenic depression leads to depletion of gonads and to pituitary changes comparable to the castration response (72, 73). Other investigators induce complete destruction of the seminiferous epithelium (without regeneration), but fail to observe a diminution of androgenic activity and castration changes in the pituitary; the copulatory performance responds to gonadotropins, although only pseudopregnancies are caused (74). AET (S, 2-amino-ethyl-iso-thiurium. Br.HBr) prevents death but does not curtail testicular injury (75); in prenatally irradiated rats (in utero), MEG counteracts the germinal effects of irradiation (76).

Specific studies of the effects of X-irradiation on the androgenic function of the testis indicate that the interstitium is markedly impaired, depending on the dose and on the age of the animal;

resistance increase with age and although the responsiveness of the Leydig cells to LH is diminished, the accessory response to exogenous androgens remains unaffected (77). Total body irradiation proves almost as damaging to the testis as topical application (78); hence, precautions in the form of shielding are recommended in investigative radiography (79).

The female reveals a paradoxical effect: prostate implants in irradiated females indicate higher levels of circulating androgens; although the follicular system is destroyed rapidly, uterine and vaginal characteristics display continued estrogen production. Signs of adrenal androgenicity being absent, the androgenic ability of the ovary seems enhanced, pointing to a shift in hormone balance, rather than to a simple decrease in estrogen production; thus the secretory pattern of irradiated ovary seems to differ quantitatively and qualitatively from that of the normal ovary (80).

Some chemical substances selectively affect testicular constituents. 7,12-dimethyl-benz(alpha)anthracene administered intravenously causes severe and selective damage to spermatogonia and resting spermatocytes (active in DNA synthesis); no other germinal elements are involved, the ovary is unaffected and other known carcinogens do not induce comparable effects (81). Cadmium (subcutaneous) damages the vascular supply of the testis resulting in necrosis of the tubules and of the interstitium; imunction on the scrotum causes eventual infertility (82). After a few weeks, the

interstitium regenerates, but one year later, 70 to 80% of the subjects possess interstitial cell tumors. The cadmium-treated animals display a higher titer of ICSH, smaller accessories and lack response to exogenous ICSH; the cadmium damage is prevented by simultaneous administration of zinc (83).

Space stresses have evoked in monkeys endocrine responses, but so far no testicular manifestations have been reported (84).

The clinicians have long recognized gonadal pathologies, some of them primary, such as agenesis, dysgenesis, destruction by trauma or tumor, all of which create a condition of primary hypogonadism; secondary hypogonadism follows to hypopituitarism by an endocrine link. Several pathological states present a picture of gonadal reduction or variable wasting secondary to metabolic, endocrine (extra-hypophyseal) or psychic disturbances; conversely, the level of endocrine sexuality affects decisively the economy of the whole organism and/or of specific regions or functions. The causal relationship is almost always unknown and the management is applied to the primary condition in the hope that the secondary situations will clear by themselves. Some of these clinical situations have been simulated in the laboratory animal.

Studies on unilateral cryptorchidized and on unilateral castrated rats, suggest that the functional integrity of the advanced germinal elements acts to regulate the pituitary secretion of FSH; the cholesterol concentration of the testis in the unilateral cryptorchid declines at first (2 days), then rapidly increases to a

maximum (16 days for the cryptorchid testis, 5 days for the scrotal one), but eventually returns to normal (30 and 9 days respectively); the total amount in both testes shows a decline at 2 days, a marked increase at 5 days and returns to normal at 16 days. Thereafter, the scrotal testis maintains normal levels, while the abdominal one gradually declines. No difference is found in the scrotal testis of the unilateral cryptorchid and in the remaining testis of the semi-castrate (85, 86). The process of testicular feminization has been shown to involve severely the interstitium (87).

The action on the testis of the peripheral nervous system is considered relatively unimportant (88), but the testis has been shown to be extremely sensitive to any derangement in the blood supply, local or hypothalamic (89). Extensive studies have revealed an influence of avitaminoses on the development and integrity of the testis (90, 91).

The diabetic patient displays sexual disturbances unrelieved by insulin management: pregnancies are rare in the female, and impotency frequent in the male, especially if diabetes has been established for 9.3 years or longer. Studies of urinary excretion show a reduction in the testosterone metabolites and an increase of adrenal conjugates. This reduction in the elimination of androsterone and etiocholanone is said to be due not to an anomaly of the testosterone metabolism, but to hypogonadotropic hypogonadism. The under-forty male responds to chorionic gonadotropin and testosterone, but

above 40, testosterone alone proves effective (92, 93, 94).

Laboratorians and clinicians are also well aware of the reverse situation, where the sex hormones act on the CBH metabolism. Large doses of estrogens induce diabetes, probably by way of adrenal stimulation following increased ACTH production caused by a high level of circulating estrogens; moderate doses attenuate the severity of diabetes resulting from pancreatectomy and estrogen therapy in the menopausal female cuts by half the insulin requirements, presumably, by inhibition of the diabetogenic factors of the pituitary. Androgens are said to intensify the diabetic state, although results are variable and the influence is also probably indirect (95).

In broad terms, everyone admits that an alteration of thyroid function brings about a modification of gonadal function. The female laboratory animal rendered hypothyroid, displays disturbances in the estrous cycles: prolonged, arrested or anovulatory; the fertility is reduced, because of lower fertilization rate, increased fetal resorption and decreased litter size. The ovary becomes more sensitive to FSH and LH of various origins, pituitary, chorionic, urinary (PMS or menopausal): large follicles are produced, but no luteinization occurs. The cretinic female possesses no vesicular follicles, no lipids, no cholesterol and increased susceptibility to cyst formation. Hyperthyroidism, irrespective of origin, is less effective: irregular or arrested cycles, decrease in ovarian responsiveness to gonadotropins (although species differences preclude extrapolation) and in the young subject, occasional arrest of follicle maturation.

The male seems less dependent than the female on thyroid function. Hypothyroidism delays sexual development in the young and the cretinic rat displays low testis weight and infantile accessories (evidently because of lack of androgens). Hyperthyroidism "impairs" reproduction, although in some species, spermatogenesis is stimulated by moderate doses of thyroid hormone (low enough to leave the thyroid unaffected). The situation in the human is nebulous; relationships are noted, but results are contradictory, in the male and in the female as well.

Data on the influence of gonads on thyroid are scarce: in the female, thyroid function is stimulated by progesterone, but inhibited by estrogens, where a prolonged treatment causes initial stimulation promptly followed by involution. The physiological enlargement of the thyroid at puberty, menstruation and pregnancy, remains unexplained. The subnormal BMR of the eunuch returns to normal with androgen therapy.

The picture of the gonad-thyroid interrelations is one of conjecture, where no cause is preferred and all situations are possible:

- 1) alteration of the response to gonadotropins: sensitization, augmentation or inhibition;
- 2) alteration in the secretion and/ release of pituitary tropins, where the thyroid hormone could exercise some control over STH, ACTH and gonadotropins;
- 3) non-specific changes in gonadal function by way of a general effect on metabolism, especially protein metabolism;
- 4) alteration in the ability of the target organs (in the sex apparatus) to respond normally in the absence or excess of thyroid hormone (88, 96-99).

Interest in environmental physiology has focused the attention of investigators on various aspects of the responses to cold stress and the establishment of a state of adaptation.

Exposition to cold elicits a rapid increase in heat production adequate for immediate maintenance of body temperature; prolonged exposure leads to adaptation or to breakdown of the thermogenesis, depending on the level of the temperature applied (100). The process of acclimation in the homeotherm represents an end-response expressing at the cellular level a delicate balance of physico-chemical factors in a highly integrated molecular pattern (101).

Early observations on acute exposure to 0°C have demonstrated the action of the adrenal cortex in resistance to stress (102), where a minor stimulus induces immediate mobilization of endogenous ACTH (103) and prolongation of stress eventually exhausts the functional reserve of the cortex (104). Regional responses to cold reveal no significant alteration in the morphology of organs, such as heart, kidney (105), but in pituitary, adrenals and thymus, a cyclic or oscillatory weight variation (with time) seems to relate with secretory phases of production, storage and release (106).

The cortical response (under pituitary control) is blocked by lesions in the posterior hypothalamus (107); functional evaluation (ascorbic acid depletion) suggests a relationship between the level of cortical response and the severity of stress, although corticosteroid production eventually returns to normal levels (108). The

possibility remains that stress might increase the responsiveness of peripheral tissues to corticosteroids, resulting in increased overall effectiveness even if the output is unchanged (109). Return to control conditions brings back steroid excretion to normal levels, although the adrenals (guinea pig) remain hypertrophic (110). Adrenal androgens known effective in immature female mice (111) add a disturbing component in the picture of the male response to cold.

Clinical and experimental studies of the physiologic effects of induced hypothermia (112-117) confirm the opportune distinction of this non-stressful condition (118) against the stress of environmental cold.

The endocrine action of cold appears to extend to the reproductive function: in the female rat, a marked increase in length of the estrous cycle follows prolonged exposure (90 days) to 2°C: the proestrous and estrous stages are increased, the metestrous and diestrous stages decreased; these changes are prevented by 50 micrograms of thyroxine daily (119).

Hibernators experience a reduction in cold tolerance during the spring rutting season, when their testes descend (118). Mature males resist less effectively than female (102) to a prolonged exposure to moderate cold (2.5°C), although their reproductive ability remains normal, in matings with control females (120). The laboratory mice as well as the house mouse (*Mus musculus*) can live and breed in the cold (0°C), provided nesting and/or huddling is allowed; the fertility is almost normal, except for a slight increase

in generation time and sibling mortality (121). The physiological adaptation of successive generations bred and reared in the cold may well include a genetic variation (122), although appreciable strain differences are observed (123). Degenerative changes fail to appear after 9-10 days of subnormal body temperature and some survivors reproduce normally (124).

Of the effect of maleness on the metabolic adjustments evoked by cold, we have a sketchy and unclear picture. At room temperature, the oxygen uptake of liver, kidney and brain slices from castrates is elevated, but returned to control levels by in vivo testosterone; the elevation of tissue respiration in castrates exposed to cold remains unaltered by in vivo testosterone (125). In vitro testosterone causes no significant modification in tissue respiration of animals at room temperature or exposed to cold, intact or castrates (126). Heat and cold act similarly on tissue respiration, in the direction of a typical stress agency (127). In summary, cold (9°C) increases oxygen consumption (by 40%) and food intake (65%); weight gains are slower and aging sets in on the basis of the metabolic rate, not of the growth rate (128).

3. Statement of intent.

In view of the conditioning action of sex and sex hormones on the stress responses, we wished to explore the reactions of the endocrine testis. To assure suitable magnification of the responses, cold was set at -5°C, a distinctly stressful level, acutely applicable, probably prohibiting adaptation in its usual

definition (129), yet allowing reasonable survival and pertinent observations on a restricted time scale; easy to apply and control, cold can also be combined with various physiological states. The present approach was selected in contradistinction to chronic exposure to moderate cold (20°C), where adaptation may be considered the common rule.

In this initial probing, where economy was desirable and in accordance with the history of Endocrinology, traditional and unsophisticated criteria were used: modification in the growth pattern, survival rates, changes in testicular function evidenced by weight changes and alterations in the histology of the testis and of the sexual accessories; associated phenomena, such as adrenal androgens and cryptorchism, were examined in their relationship and contribution to the response of the male subject.

The present investigation obeys to the avowed bias that whatever observations are made and conclusions obtained, the situation created by cold, although not a specific agency, simulates other degenerative states where the testis, exocrine or endocrine, suffers morphological and/or functional disturbances. Extrapolations could then be attempted into other areas of sexual damage, where the nature and magnitude of the stress are impossible to define and evaluate: infectious, metabolic and endocrine diseases, aging and senescence; insight could also be gained into the complex relationship between gonads and other endocrines, and a more sophisticated analysis of testicular vicissitudes would then justify the expenditure of tracer and biochemical analysis.

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Chapter One

EFFECT OF EXPOSURE TO SEVERE COLD (-5°C) ON VARIOUS ORGANS
WITH SPECIAL REFERENCE TO THE GENITAL APPARATUS
IN THE MALE ALBINO RAT.

Several investigators have associated the genital apparatus of the male with the control of body temperature. Involution of the gonads is pronounced before and during hibernation; an inverse correlation was found between hibernation and gonadal activity, as well as a "resistance" to hibernation when the gonads are active; however, hibernation continued after administration of anterior pituitary hormones, and even testosterone did not influence the continuance of hibernation. Although the gonads are the site of seasonal changes, they do not seem to exert a major influence on hibernation (1).

Application of heat to the testes for approximately 15 minutes, resulting in a local temperature of 41-45°C, caused damage to the germinal epithelium, observable after 4 hours; repair was accomplished in a period of 60 days (2). Daily application to the testes (monkeys and rats) of a similar treatment for a period of 8 weeks resulted in temporary aspermia; regeneration occurred in approximately 6 weeks (3). A similar effect was obtained in the guinea pigs and a time development was established for the depression and repair (4). Chronic exposition to out-of-door cold did not affect the sex drive of male guinea pigs (5).

Prepubertal testes resected, frozen in glycerol-saline or in saline alone at -79°C to -190°C, and then grafted in castrated adult males, retained their ability to grow and differentiate as subcutaneous or scrotal implants; variable degrees of spermatogenesis were observed as well as full maintenance of the seminal vesicles of the castrated rat (6).

Topical applications of cold (-4°C) for one hour leading to freezing of the testis, caused interruption of circulation in the testicular artery

and subsequent ischemia, resulting in severe spermatogenic damage from which regeneration may occur (7). Continuous exposure to 6°C in 50-day old rats (B.W.: 200 gms) for their entire life span, resulted in marked testicular hypoplasia: the testes were smaller than normal, displayed profound degeneration of the germinal sequence and damage to the interstitial elements (8).

Studies in our own laboratories revealed that chronic exposition (40 weeks) to moderate cold (2°C) induced a series of degenerative changes in the morphology and the physiology of the testes: cryptorchism, decrease in weight of the testes and of the sexual accessories, degeneration of the germinal sequence, higher survival rate in favor of the castrates and "toxicity" of testosterone administered to cold-exposed castrates. These alterations seemed to affect the two functional aspects of the testis: spermatogenesis and production of androgenic factors, and appeared proportional to the duration of the exposure. At 50 weeks, while the testes were still small and aspermic, the seminal vesicles returned to normal weights, revealing a new source of androgenic activity (9).

In order to analyze the response of the male genital apparatus to the stress of cold, we thought that the onset of the symptoms might be accelerated and their severity increased by a stronger stress; the temperature of -5°C was selected as a condition which would allow a sufficient period of survival for valid observations. The complex response of the organism would have to be detailed into its possible components: somatic, neural, humoral; general or topical, endocrine or exocrine.

A survey of organ responses covered the genital apparatus, some endocrine organs and some organs of purely somatic nature; the effects of cold were compared with the effects of castration, with and without testosterone therapy.

MATERIALS AND METHODS

Animal groups. - A random group of 218 male rats (Wistar Albino) was arranged as shown in Table I and surgery applied accordingly.

Table I

Distribution of animals			
Groups		20°C (N)	-5°C (F)
Absolute controls	(T)	TN (23) ¹	TF (44)
Untreated castrates	(C)	CN (63)	CF (55)
Treated castrates	(CI) ²	CIN (11)	CIF (22)

1: Group population.

2: 15-mg pellet of testosterone propionate implanted at operation time.

Surgery and Treatment. - Castration was performed by abdominal approach under light ether anesthesia. The absolute controls were not subjected to sham surgery, but were assigned the usual 5 to 7-day quarantine before entry into the cold. The implant to the treated castrates was placed subdermally between the scapulae. A minimum

post-operative period of 10 days was allowed; recovery was rapid and uneventful.

Living Quarters and Diet. - Prior to cold exposure, the animals, caged individually, lived in air-conditioned quarters maintained at approximately 20°C and were fed Purina Lab chow and tap water ad libitum. All animals entered the cold simultaneously on day Zero (13th post-operative) and were exposed to a temperature of $-5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for different periods; the rooms were ventilated, thermostatically controlled and illuminated under automatic control from 9:00 a.m. to 5:00 p.m., daily. A limited but sufficient amount of chow cubes was offered to prevent nesting. Body weights were obtained frequently (every 2-3 days) and the animals were carefully examined twice daily to observe the onset and evolution of cold injury symptoms.

The 34-day exposure was applied to a sub-group taken from the initial controls, thus providing a different age baseline (Figure 1).

Autopsy Data. - Post-mortems were performed on all animals as casualties occurred and as each exposure period was completed; organs were collected, examined, weighed wet and specimens taken for microscopic examination or Cholesterol determination. Microscopic sections were stained with Mallory trichrome for histological examination without cytological evaluation.

The pellets (Testosterone propionate - 15 mg) were kindly provided by the Ciba Company, Montreal, through the courtesy of Dr. C.W. Murphy.

Cholesterol was extracted (10), plated as digitonide (11) on filter paper and weighed as total cholesterol. Residual testosterone pellets were recovered and when judged in good shape, dried and weighed.

Statistics. - All values were screened by means of the Dixon test for extreme values (12); the Analysis of Variance was performed according to the "R X 2 Tables" of Yates (13) complemented by the Students' T and/or the "Multiple Range Test" of Duncan (14). All tables quote the arithmetical mean with the standard error of the mean. Results are stated for each exposure period without time-axis development. Since no significant difference appeared between data from the casualties and from the sacrificed, the data were pooled and made to represent the "class" results.

RESULTS

1. Body Weights (Table II)

The pre-exposure body weights show some difference resulting 1) from the difficulty in synchronizing large animal groups, 2) from the growth retardation caused by the surgical trauma, and 3) to some extent, by the new physiological status. Although significant, this initial difference is partially resorbed in the course of the experiment, as the rate of growth of all castrates appears normal; however, the initial setback is never compensated.

The terminal body weights manifest the effect of ^{the} age and normal growth among the groups maintained at room temperature; the effect of cold (expressed as % difference from the respective control) appears rather consistently as an arrest of growth and a definite loss of body weight, probably proportional to the duration of exposure. The untreated castrate shows less regression due to cold than the treated castrate.

The growth curves (Figure 1) illustrate the effect of cold per se exclusive of any influence from the physiological situation.

Table II

Body weights (grams)				
Groups	Initial	Terminal		
		18 days	24 days	34 days
Absolute controls				
(20°C)	267.4 ± 4.1 ¹ (23) ²	331.5 ± 6.8 (10)	345.8 ± 10.7 (9)	409.2 ± 8.1 (4)
(-5°C)	266.6 ± 3.4 (44)	216.2 ± 5.7 (20) <u>-35%</u> (30) ³	223.0 ± 5.6 (18) <u>-36%</u> (27)	230.3 ± 7.3 (6) <u>-44%</u> (10)
Untreated castrates				
(20°C)	246.1 ± 2.3 (63)	299.4 ± 6.6 (11)	321.6 ± 8.3 (18)	385.3 ± 3.9(34)
(-5°C)	240.0 ± 3.0 (55)	203.0 ± 4.7 (20) <u>-32%</u> (31)	243.5 ± 5.8 (19) <u>-27%</u> (37)	263.7 ± 4.7(16) <u>-32%</u> (50)
Treated castrates				
(20°C)	229.2 ± 6.9 (11)	300.6 ± 9.5 (11)	-----	-----
(-5°C)	232.1 ± 3.2 (22)	193.8 ± 4.8 (21) <u>-37%</u> (32)	-----	-----

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

---: p < 0.001

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
I. Initial body weights:	t = 6.3 p < 0.001	N.S.	N.S.
II. Terminal body weights:			
1) 18-day period:	N.S.	t = 17.9 p < 0.001	N.S.
2) 24-day period:	N.S.	t = 11.7 p < 0.001	**
3) 34-day period:	t = 2.48 p < 0.02	t = 20.4 p < 0.001	N.S.

2. Perirenal fat. (Table III) The observations on body weight changes are further illustrated by data on the perirenal fat; although the dissection is hazardous, the fatty mass extending from the sub-phrenic area down along the dorsal wall of the abdominal cavity, investing kidneys, adrenals and spleen and reaching into the pelvic cavity, was assumed to represent the so-called "perirenal fat". In the room-temperature animals, this fatty mass represents approximately 1% of the total body weight; the cold-exposed animals fall close to a single value of 0.3% (15).

Table III

Perirenal fat (grams) 18-day exposure				
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	3.24 ± 0.20 ¹ (9) ²	0.68 ± 0.07 (12)	<u>-79%</u> (21) ³
Untreated castrates	(C)	3.14 ± 0.30 (11)	0.61 ± 0.20 (4)	<u>-81%</u> (15)
Treated castrates	(CI)	4.82 ± 0.60 (11)	0.57 ± 0.12 (13)	<u>-88%</u> (24)

1: Standard error of the mean.

 : p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	2	8.7	4.4	1.04	N.S.
Effect of cold	1	123.8	—	29.56	**
Interaction	2	10.4	5.2	1.24	N.S.
Error	54	227.2	4.19		

Effect of cold: t = 10.86 p < 0.001

3. Signs and Symptoms of Cold Injury.

A recital of these manifestations may be repetitious, but will serve as illustration of cold-induced injury. The symptoms usually develop in the following order: 1) swelling of the paws, especially the hind paws, 2) reddening of the penis with priapism presumably of mechanical origin due to coagulation of the prostatic secretion, occasionally resulting in urethral stricture, 3) necrosis of the tail with or without self-inflicted amputation.

In the untreated controls (TF), the onset of the symptoms is rapid and involves a relatively large number of animals; recovery is infrequent, and the animals become stationary in their mangy appearance. The testosterone-treated castrates (CIF) show a fulminating onset of symptoms involving practically the whole population; the symptoms evolve very rapidly, without any instance of recovery; the high incidence of mortality follows the level of morbidity. In these two groups, a severe bladder obstruction with a secondary nephrosis and a well marked xerophthalmia have been encountered occasionally; in one case, lithiasis of the kidney was characteristic. Among the untreated castrates (CF), the level of morbidity is low; the onset of the symptoms is delayed and a smaller number of subjects is involved; recovery occurs after symptoms of subacute intensity. The animals maintain longer, a better general appearance: paws, nose, tail and fur.

Some animals of the (CIF) group were by mistake extensively shaven prior to pellet implantation, but the added exposure resulting from the denuded area of the back did not affect survival nor alter any of the

other responses.

4. Survival Rates. (Table IV)

All the animals at room temperature survived through the experiment. Among the cold-exposed, the treated castrates offered the least resistance in that they showed a higher level of morbidity and greater mortality at an earlier date; a 50% survival was maintained for only 16 days, when the group had to be discontinued. The unoperated controls were next to succumb, but their overall survival was not far different from that of the untreated castrates; this latter group showed last the inception of breakdown as well as an overall lower level of morbidity.

Table IV

Groups	Survival rates (% of initial population)					
	Days					
	0	8	16	20	24	36
Absolute controls (TF)	100	99	90	86	75	64
Untreated castrates (CF)	100	96	88	85	76	68
Treated castrates (CIF)	100	86	50	45	--	--

5. Organ Weights and Other Data.

(1) Testes (Table V).

A slight non-significant increase due to age appears among the controls at room temperature. In the cold-exposed animals, all testes are found in abdominal position and present the usual characteristics: pale, soft, reduced in size and apparently ischemic. The weight loss is always significant, proportional to the duration of the exposure, but unrelated to chronological age.

Table V

Groups	Testes (weight in grams)		
	Duration of exposure to cold (-5°C)		
	18 days	24 days	34 days
Absolute controls (20°C)	3.13 ± 0.06 ¹ (10) ²	3.25 ± 0.11 (9)	3.43 ± 0.04 (3)
Cold-exposed (-5°C)	2.52 ± 0.12 (20) <u>-20%</u> (30) ³	2.67 ± 0.11 (18) <u>-15%</u> (27)	2.02 ± 0.30 (6) <u>-31%</u> (9)

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

—: p < 0.01

—: p < 0.001

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F obs.	
Effect of age	2	0.4172	0.2086	0.88	N.S.
Effect of cold	1	8.34	8.34	35.34	**
Interaction	2	1.148	0.574	2.43	N.S.
Error	60	14.154	0.236		

Effect of cold t = 6.70 p < 0.001

The same time relationship applied to the Total Cholesterol contents of the testis (Table VI), where an ^{apparent} elevation (relative to the control) is observed as the duration of the exposure increases; the room-temperature controls, on the contrary, show a decrease (non-significant) of total cholesterol as age advances.

Table VI

Groups	Total testis cholesterol (mg % grams of wet tissue)		
	Duration of exposure to cold (-5°C)		
	18 days	24 days	34 days
Absolute controls (20°C)	140.9 ± 2.0 ¹ (10) ²	128.2 ± 11.8 (9)	101.3 ± 12.2 (3)
Cold-exposed (-5°C)	159.3 ± 10.8 (20) +11% (30) ³	178.9 ± 13.9 (18) +28% (27)	151.7 ± 6.3 (5) +34% (8)

1: Standard error of the mean. _____: $p < 0.01$

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of age	2	4233.8	2161.9	1.09	N.S.
Effect of cold	1	16789.4	16789.4	8.43	* *
Interaction	2	3750.6	1875.3	0.94	N.S.
Error	59	117448.8	1990.7		

Effect of cold $t = 3.16$ $p < 0.01$

At post-mortem, a number of pellets were recovered from the implantation site (Table VII); the difference between the original weight of 15 mg and the dry weight of the residual pellet was assumed to represent the amount of testosterone "utilized" in the replacement therapy. If the daily rate of utilization is calculated, a slight difference (12%) distinguishes the cold-exposed from the normal-temperature subjects; if however, the "daily consumption" is estimated in relation to the body weight (terminal), the consumption in the cold becomes twice that observed at room temperature.

Table VII

Testosterone replacement (amount utilized from original 15-mg implant)		
	20°C (CIN)	-5°C (CIF)
Average post-implantation period (days)	33	28
Recovered pellet residue (weighed dry - mg)	6.0 ± 0.1 ¹ (9) ²	6.4 ± 0.4 (9)
Total utilized (mg)	9.0	8.6
Estimated daily utilization (milligrams)	0.27	0.31

¹: Standard error of the mean.

²: Number of pellets recovered in reliable state.

(2) Sexual accessories (Tables VIII and IX).

Inspection of the organ weights suggests the following remarks.

- a) The effectiveness of testosterone replacement is unaffected by exposure to cold;
- b) The changes due to cold among the treated castrates are assumed to represent the resultant of the depression due to cold vying with the increase due to testosterone treatment;
- c) The weight decrease due to cold per se in the absolute controls is highly significant, consistent and in direct relationship with the duration of exposure, irrespective of the chronological age;
- d) After a certain period of exposure, the accessories of the cold-exposed castrates (especially prostate) begin to show a slight increase in weight;
- e) The analysis of variance is sometimes hampered (e.g. 24-day period) by the buffer effect of divergent results and by the broad dispersion of the values under study.

Table VIII

		Prostate (weight in milligrams)		
		Duration of exposure to cold (-5°C)		
Groups		18 days	24 days	34 days
Absolute controls	(20°C)	510.0 ± 40.0 ¹ (10) ²	475.6 ± 21.5 (9)	525.0 ± 3.5 (2)
	(-5°C)	210.0 ± 20.0 (20)	190.8 ± 25.3 (18)	131.9 ± 28.5 (6)
		<u>-59%</u> (30) ³	<u>-60%</u> (27)	<u>-75%</u> (8)
Untreated castrates	(20°C)	80.0 ± 10.0 (11)	19.1 ± 1.6 (16)	16.2 ± 1.1 (34)
	(-5°C)	31.9 ± 4.0 (16)	22.4 ± 2.8 (18)	18.0 ± 1.8 (16)
		<u>-62%</u> (27)	+15% (34)	+10% (50)
Treated castrates	(20°C)	660.0 ± 40.0 (11)	----	----
	(-5°C)	580.0 ± 40.0 (19)	----	----
		-12% (30)		

1: Standard error of the mean.

==: p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
1) 18-day exposure	<u>CI</u> <u>T</u> <u>C</u>	t = 2.17 p < 0.05	N.S.
2) 24-day exposure	t = 8.38 p < 0.001	N.S.	* *
3) 34-day exposure	t = 3.33 p < 0.01	t = 16.5 p < 0.001	* *

Table IX

		Seminal vesicles (weight in milligrams)		
		Duration of exposure to cold (-5°C)		
Groups		18 days	24 days	34 days
Absolute controls	(20°C)	620.0 ± 30.0 ¹ (10) ²	571.1 ± 24.3 (9)	666.7 ± 42.4 (3)
	(-5°C)	240.0 ± 20.0 (20)	227.0 ± 24.9 (18)	243.1 ± 43.6 (6)
		<u>-61%</u> (30) ³	<u>-60%</u> (27)	<u>-65%</u> (9)
Untreated castrates	(20°C)	80.0 ± 6.0 (11)	57.5 ± 2.0 (18)	44.5 ± 1.2 (34)
	(-5°C)	60.0 ± 4.0 (19)	61.2 ± 2.9 (19)	40.7 ± 1.5 (16)
		<u>-25%</u> (30)	+6% (37)	-9% (50)
Treated castrates	(20°C)	810.0 ± 30.0 (11)	-----	-----
	(-5°C)	840.0 ± 40.0 (16)	-----	-----
		+4% (27)		

1: Standard error of the mean.

___: p < 0.01

2: Group population.

3: Aggregate population.

====: p < 0.001

Analysis of variance - summary

	Effect of treatment		Effect of cold	Interaction
1) 18-day exposure	<u>CI</u>	<u>T C</u>	t = 2.14 p < 0.05	N.S.
2) 24-day exposure	t = 7.63	p < 0.001	N.S.	**
3) 34-day exposure	t = 4.29	p < 0.001	N.S.	**

(3) Stress Indicators (Tables X - XII)

Castration per se causes "feminization" of the adrenals (+24%) and return of the thymus to juvenile values (+30%); testosterone cancels this effect and returns the weight values to adult or even senile levels. Cold per se causes an increase in adrenal weight in the absolute control and in the treated castrate, but cancels the feminoid values in the untreated castrate; a prolonged exposure (34 days) elicits in the adrenal of the castrate a normal response.

Table X

Adrenals (weight in milligrams)				
Groups	Duration of exposure to cold (-5°C)			
	18 days	24 days	34 days	
Absolute controls	(20°C) 39.8 ± 2.2^1 (10) ²	45.0 ± 2.4 (9)	39.3 ± 0.2 (3)	
	(-5°C) 47.0 ± 1.2 (20)	52.7 ± 2.0 (18)	53.3 ± 4.5 (6)	
	<u>+15%</u> (30) ³	<u>+14%</u> (27)	<u>+26%</u> (9)	
Untreated castrates	(20°C) 50.6 ± 4.0 (11)	59.0 ± 2.8 (18)	52.3 ± 1.4 (34)	
	(-5°C) 52.7 ± 1.9 (20)	57.8 ± 2.6 (19)	68.0 ± 3.5 (16)	
	<u>+4%</u> (31)	<u>-2%</u> (37)	<u>+23%</u> (50)	
Treated castrates	(20°C) 36.7 ± 1.7 (11)	----	----	
	(-5°C) 43.9 ± 1.4 (18)	----	----	
	<u>+16%</u> (29)			

1: Standard error of the mean.

--: $p < 0.05$

2: Group population.

---: $p < 0.01$

3: Aggregate population.

====: $p < 0.001$

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
1) 18-day exposure	N.S.	$t = 2.79$ $p < 0.01$	N.S.
2) 24-day exposure	N.S.	N.S.	N.S.
3) 34-day exposure	N.S.	$t = 3.66$ $p < 0.001$	N.S.

Total adrenal cholesterol (Table XI) shows no statistically significant change due to castration and/or testosterone replacement, although the trends observed could be biologically interesting (16); cold per se causes a significant increase except in the untreated castrate.

Table XI

Total adrenal cholesterol (mg % gms of wet tissue) 18-day exposure				
Groups	20°C (N)	-5°C (F)	Difference	
Absolute controls (T)	877 ± 73 ¹ (7) ²	1457 ± 138 (11)	+40% (18) ³	
Untreated castrates (C)	1347 ± 165 (7)	1602 ± 220 (13)	+15% (20)	
Treated castrates (CI)	1282 ± 225 (8)	2121 ± 258 (8)	+40% (16)	

1: Standard error of the mean.

—: p < 0.05

2: Group population.

3: Aggregate population.

—: p < 0.01

Analysis of variance - summary

Effect of treatment	N.S.
Effect of cold	t = 3.33 p < 0.01
Interaction	N.S.

The thymolysis due to cold gives a clear picture of the duration and intensity of the stress: the thymus responds rather uniformly among the various groups to a given period of exposure and the level of the response appears proportional to the magnitude of the stress, irrespective of the physiological status.

Table XII

		Thymus (weight in grams)		
		Duration of exposure to cold (-5°C)		
Groups		18 days	24 days	34 days
Absolute controls	(20°C)	0.15 ± 0.03 ¹ (10) ²	0.14 ± 0.03 (9)	0.38 ± 0.01 (3)
	(-5°C)	0.20 ± 0.03 (19)	0.12 ± 0.02 (18)	0.06 ± 0.003 (5)
		<u>-55%</u> (29) ³	<u>-72%</u> (27)	<u>-84%</u> (8)
Untreated castrates	(20°C)	0.60 ± 0.05 (11)	0.72 ± 0.04 (18)	0.56 ± 0.02 (34)
	(-5°C)	0.17 ± 0.02 (18)	0.28 ± 0.01 (19)	0.17 ± 0.07 (16)
		<u>-72%</u> (29)	<u>-61%</u> (37)	<u>-70%</u> (50)
Treated castrates	(20°C)	0.22 ± 0.02 (10)	-----	-----
	(-5°C)	0.08 ± 0.01 (12)	-----	-----
		<u>-64%</u> (22)		

1: Standard error of the mean.

 : p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
1) 18-day exposure	N.S.	t = 6.92 p < 0.001	* *
2) 24-day exposure	t = 7.71 p < 0.001	t = 10.0 p < 0.001	*
3) 34-day exposure	t = 3.83 p < 0.001	t = 14.4 p < 0.001	N.S.

(4) Endocrine Organs.

I. Pituitary (Table XIII). Castration causes a considerable increase in weight (+26%), while testosterone brings back these values to normal. Cold per se induces a notable weight depression (-31%), fairly uniform among all groups and consistently significant; this effect appears related to the duration of exposure irrespective of age.

Table XIII

		Pituitary (weight in milligrams)			
		Duration of exposure to cold (-5°C)			
Groups		18 days	24 days	34 days	
Absolute controls	(20°C)	10.4 ± 0.7 ¹ (10) ²	10.1 ± 0.6 (7)	11.4 ± 1.1 (3)	
	(-5°C)	7.5 ± 0.2 (20)	8.1 ± 0.5 (17)	6.8 ± 0.1 (4)	
		<u>-28%</u> (30) ³	<u>-20%</u> (24)	<u>-40%</u> (7)	
Untreated castrates	(20°C)	14.5 ± 0.6 (9)	14.6 ± 0.3 (17)	-----	
	(-5°C)	10.0 ± 1.3 (20)	11.7 ± 0.3 (17)	-----	
		<u>-31%</u> (29)	<u>-20%</u> (34)		
Treated castrates	(20°C)	9.5 ± 0.3 (9)	-----	-----	
	(-5°C)	6.8 ± 0.2 (19)	-----	-----	
		<u>-34%</u> (28)			

1: Standard error of the mean.

--: p < 0.05

2: Group population.

---: p < 0.01

3: Aggregate population.

====: p < 0.001

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
1) 18-day exposure	N.S.	t = 5.61 p < 0.001	N.S.
2) 24-day exposure	t = 10.0 p < 0.001	t = 5.48 p < 0.001	N.S.
3) 34-day exposure	---	t = 4.17 p < 0.01	---

II. Thyroid (Table XIV). The changes in weight were explored in one exposure period only; castration with or without testosterone reveals no significant effect. Cold however, induces a rather uniform and significant weight depression.

Table XIV

Thyroid (weight in milligrams) 18-day exposure				
Groups	20°C (N)	-5°C (F)	Difference	
Absolute controls (T)	21.0 ± 0.9 ¹ (10) ²	17.4 ± 1.0 (20)	-17%	(30) ³
Untreated castrates (C)	17.6 ± 0.3 (9)	15.9 ± 0.8 (19)	-10%	(28)
Treated castrates (CI)	18.8 ± 0.5 (10)	16.0 ± 0.7 (19)	-15%	(29)

- 1: Standard error of the mean. _ _ : p < 0.05
 2: Group population. _ _ : p < 0.01
 3: Aggregate population.

Analysis of variance - summary

Effect of treatment	N.S.
Effect of cold	t = 4.09 p < 0.001
Interaction	N.S.

(5) A few non-endocrine organs were selected: the liver as a barometer of the nutritional state of the animal, the spleen as an indicator of traumatic response and the lungs, heart and kidneys as purely somatic organs.

I. Liver (Table XV). No appreciable difference appears due to age or to physiological condition. Cold however induces a significant weight decrease parallel to the pattern of losses in body weight and perhaps reflecting the overall body emaciation.

Table XV

		Liver (Weight in grams)		
		Duration of exposure		
Groups		18 days	24 days	34 days
Absolute controls	(20°C)	13.7 ± 0.7 ¹ (10) ²	11.7 ± 0.5 (8)	12.4 ± 0.06 (2)
	(-5°C)	11.6 ± 0.2 (18)	9.9 ± 0.4 (18)	10.9 ± 0.6 (6)
		<u>-15%</u> (28) ³	<u>-16%</u> (26)	<u>-12%</u> (8)
Untreated castrates	(20°C)	11.9 ± 0.5 (10)	12.1 ± 0.6 (18)	----
	(-5°C)	9.5 ± 0.4 (19)	11.8 ± 0.3 (19)	----
		<u>-20%</u> (29)	<u>-3%</u> (37)	
Treated castrates	(20°C)	13.3 ± 0.6 (11)	----	----
	(-5°C)	9.6 ± 0.6 (19)	----	----
		<u>-30%</u> (30)		
1: Standard error of the mean		—: p < 0.05		
2: Group population		—: p < 0.01		
3: Aggregate population		—: p < 0.001		

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
1) 18-day exposure	N.S.	t = 6.00 p < 0.001	N.S.
2) 24-day exposure	t = 3.33 p < 0.01	t = 2.20 p < 0.05	N.S.
3) 34-day exposure	----	t = 2.50 p < 0.05	----

II. Spleen (Table XVI). A weight depression is observed due to cold only; this depression appears similar to the response of the spleen against a serious assault on the circulatory system.

Table XVI

Spleen (weight in grams) 18-day exposure				
Groups	20°C (N)	-5°C (F)	Difference	
Absolute controls (T)	0.58 ± 0.02 ¹ (9) ²	0.35 ± 0.02 (19)	-1.0%	(28) ³
Untreated castrates (C)	0.66 ± 0.04 (11)	0.28 ± 0.02 (18)	-58%	(29)
Treated castrates (CI)	0.55 ± 0.03 (11)	0.23 ± 0.02 (18)	-58%	(29)

1: Standard error of the mean —: p < 0.001

2: Group population

3: Aggregate population

Analysis of variance - summary

Effect of treatment N.S.

Effect of cold t = 19.5 p < 0.001

Interaction N.S.

III. Lungs (Table XVII). The physiological status does not seem to cause any observable effect on the weight of the lungs; cold causes a moderate and consistent weight loss. The incidence of respiratory involvements or infections is not different from that observed among the control animals

at room temperature. The combination of cold and testosterone brings about a curious and significant increase in weight, but in the absence of microscopic information, no discussion is offered.

Table XVII

		Lungs (weight in grams)			
		Duration of exposure			
Groups		18 days	24 days	34 days	
Absolute controls	(20°C)	1.36 ± 0.05 ¹ (9) ²	1.32 ± 0.04 (9)	1.47 ± 0.0 (2)	
	(-5°C)	1.18 ± 0.03 (19)	1.31 ± 0.02 (18)	1.31 ± 0.04 (6)	
		<u>-13%</u> (28) ³	-1% (27)	<u>-11%</u> (8)	
Untreated castrates	(20°C)	1.34 ± 0.06 (11)	1.46 ± 0.04 (17)	----	
	(-5°C)	1.16 ± 0.03 (17)	1.30 ± 0.03 (19)	----	
		<u>-13%</u> (28)	<u>-9%</u> (36)	----	
Treated castrates	(20°C)	1.22 ± 0.06 (11)	----	----	
	(-5°C)	1.67 ± 0.11 (19)	----	----	
		<u>+27%</u> (30)			

1: Standard error of the mean

--: p < 0.05

2: Group population

---: p < 0.01

3: Aggregate population

====: p < 0.001

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
1) 18-day exposure	N.S.	N.S.	* *
2) 24-day exposure	N.S.	t = 2.75 p < 0.01	* *
3) 34-day exposure	----	t = 4.00 p < 0.01	-----

IV. Heart and kidneys (Tables XVIII-XX). Organ weights display no change whatever, due to cold or to physiological status.

The values for heart cholesterol (Table XX) appear labile. At room temperature, the decreasing order is as follows:

Treated castrate > Control > Untreated castrate; in the cold, the order is reversed:

Untreated castrate > Control > Treated castrate. The low grade of significance of these data does not allow speculation on these findings.

Table XVIII

Heart (weight in grams) 18-day exposure				
Groups	20°C (N)	-5°C (F)	Difference	
Absolute controls (T)	0.90 ± 0.04 ¹ (10) ²	0.86 ± 0.02 (20)	-4% (30) ³	
Untreated castrates (C)	0.84 ± 0.03 (11)	0.84 ± 0.02 (19)	±0% (30)	
Treated castrates (CI)	0.89 ± 0.03 (11)	0.88 ± 0.02 (19)	-1% (30)	

1: Standard error of the mean

2: Group population

3: Aggregate population

Analysis of variance - summary

Effect of treatment	N.S.
Effect of cold	N.S.
Interaction	N.S.

Table XIX

Kidneys (weight in grams) 18-day exposure			
Groups	20°C (N)	-5°C (F)	Difference
Absolute controls (T)	2.56 ± 0.07 ¹ (9) ²	2.48 ± 0.08 (20)	-4% (29) ³
Untreated castrates (C)	2.26 ± 0.05 (11)	2.24 ± 0.05 (19)	-2% (30)
Treated castrates (CI)	2.57 ± 0.10 (11)	2.56 ± 0.09 (19)	-1% (30)

1: Standard error of the mean

2: Group population

3: Aggregate population

Analysis of variance - summary

Effect of treatment N.S.

Effect of cold N.S.

Interaction N.S.

Table XX

Total heart cholesterol (mg % gms of wet tissue) 18-day exposure			
Groups	20°C (N)	-5° C (F)	Difference
Absolute controls (T)	116 ± 26 ¹ (7) ²	115 ± 11 (12)	-1% (19) ³
Untreated castrates (C)	110 ± 5 (7)	126 ± 6 (11)	+13% (18)
Treated castrates (CI)	130 ± 18 (8)	85 ± 7 (15)	-35% (23)

1: Standard error of the mean

-- : p < 0.05

2: Group population

3: Aggregate population

Analysis of variance - summary

Effect of treatment N.S.

Effect of cold N.S.

Interaction N.S.

(6) Water contents of various organs (Table XXI). As a quick check on possible fluid or ion movements due to cold or to physiological conditions, the water contents of a number of organs was determined. Of all the organs tested, only the prostate showed a significant change due to castration per se and unrelated to cold. In all the other organs, the water contents appeared as a constant value probably characteristic of the organ itself and unaffected by physiological status or stress response.

% Water contents of various organs

Organs	Groups		Duration of exposure	
			24 days	34 days
Prostate	Controls	(20°C)	80.6 ± 1.1 ¹ (8) ²	81.2 ± 0.6 (3)
		(-5°C)	83.0 ± 0.7 (17)	82.6 ± 1.5 (5)
			+2% (25) ³	+2% (8)
	Castrates	(20°C)	66.5 ± 0.5 (18)	----
		(-5°C)	63.3 ± 3.5 (18)	----
			-5% (36)	
Analysis of variance - summary				
	Effect of treatment	t = 5.77	p < 0.001	----
	Effect of cold	N.S.		N.S.
	Interaction	N.S.		----
Seminal vesicles	Controls	(20°C)	76.8 ± 1.1 (8)	79.0 ± 1.4 (3)
		(-5°C)	78.8 ± 0.4 (16)	78.8 ± 0.7 (5)
			+2% (24)	±0% (8)
	Castrates	(20°C)	75.9 ± 0.8 (18)	----
		(-5°C)	78.1 ± 0.4 (18)	----
			+2% (36)	
No significant difference				
Adrenals	Controls	(20°C)	71.9 ± 0.5 (8)	69.2 ± 0.9 (3)
		(-5°C)	75.3 ± 0.8 (16)	68.8 ± 1.7 (5)
			+4% (24)	-1% (8)
	Castrates	(20°C)	73.9 ± 0.1 (18)	----
		(-5°C)	73.1 ± 0.6 (18)	----
			-1% (36)	
No significant difference				
Thymus	Controls	(20°C)	79.0 ± 0.5 (9)	80.0 ± 0.0 (2)
		(-5°C)	80.0 ± 1.3 (16)	77.9 ± 1.4 (6)
			+1% (25)	-3% (8)
	Castrates	(20°C)	79.1 ± 0.4 (18)	----
		(-5°C)	80.7 ± 0.9 (19)	----
			+2% (37)	
No significant difference				
Liver	Controls	(20°C)	69.4 ± 0.9 (9)	69.5 ± 1.6 (3)
		(-5°C)	72.8 ± 0.8 (18)	74.1 ± 0.7 (6)
			+5% (27)	+6% (9)
	Castrates	(20°C)	72.2 ± 0.8 (16)	----
		(-5°C)	72.4 ± 0.7 (18)	----
			±0% (34)	
No significant difference				
Lungs	Controls	(20°C)	77.9 ± 0.3 (9)	79.5 ± 0.8 (3)
		(-5°C)	78.2 ± 0.2 (17)	79.2 ± 0.3 (5)
			±0% (26)	±0% (8)
	Castrates	(20°C)	78.2 ± 0.4 (17)	----
		(-5°C)	78.6 ± 0.4 (19)	----
			±0% (36)	
No significant difference				

1: Standard error of the mean

2: Group population

6. Microscopic observations.

1. The testes.

Inspection of Plate I reveals progressive damage to the tubules and germinal sequence; at 18 days (Fig. 2), spermatids have begun clumping giving rise to "berries", mechanical disorganization of the sequence and pycnosis of a number of germinal elements, are observable. At 24 days (Fig. 3), reduction of the tubular diameter is evident and the number of quiescent tubules appears greater than normal. At 34 days, (Fig. 4) the "berries" resulting from consolidation or from aberrant division invade the tubular field; some tubules are still active, but a significant number display complete arrest usually between the cyte-I and cyte-II stages.

The evaluation of the damage to the interstitial spaces is near to impossible; some observers have seen a hypertrophy of the Leydig triangles but this alteration is fictitious, merely resulting from the change in the relative size of the tubules. Pycnosis of the nuclei, vacuolization of the cytoplasm, shrinking of the cells have been cited as proof of anatomical damage, but the present data do not provide clear anatomical signs of functional alterations.

2. Adrenals (Plates II and III).

1. Effect of age. Figures 5, 7 and 9 show controls at different ages: no obvious difference appears due to age per se.

2. Effect of castration (Figures 11 and 13). The well-known hypertrophy of the zona fasciculata is observed with loss of sudanophilia in the glomerulosa. Testosterone treatment (Fig. 15) accentuates the sudanophobia and reduces the thickness of the fasciculata to control levels.

3. Effect of cold (Figures 6, 8, 10). Cold per se causes depletion of sudanophilic material in the reticularis and hypertrophy of the fasciculata. In the castrate (Fig. 12, 14) the sudanophobia due to cold is accentuated and the thickness of the fasciculata is approximately normal. Testosterone treatment (Fig. 16) superimposed to cold depletes sudanophilic material and shows the normal features of the cold-exposed adrenal (Fig. 6).

DISCUSSION

1. Effect of castration

(1) Somatic aspects. The rate of growth appears quite normal, although the initial setback due to surgery is never compensated and perhaps aggravated (non-significantly) by testosterone treatment. Perirenal fat shows a slight (non-significant) variation possibly associated with the physiological condition (15); 1.6% of the body weight in the treated castrates, 1% in the untreated castrates and 0.97% in the control (16). The somatic organs examined display no effect ascribable to castration and heart values demonstrate no cholesterol mobilization.

(2) Endocrine organs. The changes in organ weight and histological picture (adrenal) confirm the classical observations on the effects of castration: "feminization" of the adrenals, juvenile values of thymus weight. The thyroid is slightly depressed and the pituitary slightly elevated: these changes are cancelled by testosterone treatment. The cholesterol content of the adrenal rises slightly (not significantly) although sudanophilia appears reduced.

(3) Sexual accessories. The degeneration of these adnexa after castration is an established fact of endocrinology, as well as their restoration by testosterone therapy to a value consistent with some homeostatic level (17). Water content changes only in the prostate and because of castration exclusively.

2. Effect of cold

(1) Somatic aspects

I. Body weights. The loss of body weight appears not only as a retardation of growth, but as a distinct regression below the initial weight level; this loss might well represent the metabolic debt incurred shortly after the initiation of the cold stress (18), never to be compensated and proportionately aggravated by a prolongation of exposure. Among the somatic structures affected by cold, the muscle mass is said to be the most important (19). Perirenal fat was used as an index of somatic changes; this accumulation of intra-abdominal fat is a reliable index of body maintenance and total fat over a wide range of values (20), and is independent of diet (21). We have found it a good indicator of the stress response: the treated castrate seems to accumulate more fat at room temperature and to lose proportionately more in the cold. This wasting of the body reserves follows closely the pattern of weight losses and the trends observed in the survival rates.

II. Survival. The cold-exposed castrate survives as well as the control, but the testosterone-treated castrate is definitely in an inferior situation: it appears that exogenous testosterone becomes toxic if administered to a cold-stressed castrate. If this view is correct, then the better status of the castrate would result from the absence of the main source of androgens.

III. Testosterone utilization. The weight of the residual pellet extracted at post-mortem from the treated castrates, leads us to believe

that the amount utilized and the rate of utilization are fairly uniform and unaffected by cold. Our data allow only moderate speculation, but we feel that the observed rate of utilization of exogenous testosterone would be consistent with a certain level of body needs representing endocrinological (androgenic) homeostasis (17). Why then should therapeutic testosterone prove more toxic than the androgens normally produced in the intact animal, by the testes and the adrenals? Or is the severe depression of androgen production in the cold-exposed control the ransom of adaptation and survival?

IV. Somatic organs. The weight of the liver is depressed, markedly in the treated castrate, moderately in the other groups; thus a nutritional "barometer" would remain unaffected by the duration of exposure. The weight of the spleen is depressed uniformly in all groups, in the manner of a circulatory shock response. The weight of the lungs is moderately depressed in all groups, except in the treated castrates, where an unexplained elevation is observed. The weight of heart and kidneys is unaffected.

The observed changes cannot be ascribed to a change in organ composition; indeed, the water contents is unaffected by cold and appears as a value characteristic of the organ concerned. Thus, the value of this functional criterion is highly debatable. The present data do not conform with other observations, where hypertrophy of liver and kidney has been observed (19, 22, 27); only after a shorter period of exposure have we observed a slight hypertrophy of

some somatic organs (24). We feel that the interpretative value of weight changes in somatic organs begs further elucidation.

(2) Endocrine organs. The response of adrenals and thymus in relation to duration and intensity of stress is well known in cold physiology. The hypertrophy of the adrenal is assumed to represent an enhancement of cortical function (25). The increase in size was sustained and did not show tendency to revert to normal, as adaptation (18) was assumed impossible under the present conditions. In the castrate, the feminoid characters of the adrenals are lost and after a suitable period, the adrenals display a normal relative increase. The significant increase in total adrenal cholesterol could be interpreted as resulting from a partial block to normal biosynthesis of adrenal steroids causing an accumulation of cholesterol as precursor, or as an increased steroid biosynthesis where more precursor becomes available, or as a switch in pathways of steroid production. On the other hand, reduced sudanophilia suggests a decrease in local steroids and an increase in mobilization; however, the heart cholesterol does not vary and would argue against any firm conclusion on this complex question.

Pituitary and thyroid show a weight depression, irrespective of age; does this mean increased delivery of specific products or functional depression? The second alternative seems unlikely, at least for the thyroid, and a clear answer must await physiological and histochemical evaluation.

(3) The Testes.

I. Spermatogenic function. Hypoplasia of the seminiferous tubules has

been shown to result from chronic exposure to moderate cold (8,9); a comparable regression is now observed after acute exposition to a more severe cold, indicating a relationship between the extent of testicular damage, the duration of exposure and the severity of the applied stress. The loss of weight and the anatomical damage to the germinal sequence are not as severe as those observed in the cryptorchid, spontaneous or experimental (26, 27), although the testis in the present situation, becomes abdominal in all exposed animals shortly after entry into the cold room.

The damage involves the more differentiated elements; no cytological count or evaluation of cellular associations (28, 29) was attempted and the topical temperature of the testis was not measured. However, the histological damage is typical enough to differentiate the cold-exposed testis from the control organ and to represent qualitatively the duration of exposure. Thus, these damages could be ascribed to a combination of conditions brought about by exposure to cold: cryptorchid position in part, perhaps compensated by a lowered body temperature, overall loss of body weight, metabolic and/or circulatory impairments.

II. Endocrine function. The interstitial spaces are taken to display some damage, but evaluation is almost impossible. All observations remain uncertain in the absence of a safe visual criterion of functional level; an indirect evaluation of endocrine activity (androgenic) must therefore be found elsewhere.

The sexual accessories, prostate and seminal vesicles, have always been considered reliable indicators of androgenic activity (31, 32, 33, 34)

because of their direct relationship to the titer of circulating androgens (35). The accessories in the cold-exposed control show a level of regression comparable to that observed in the castrate at room temperature. In the cold-exposed castrate, cold per se has but a slight topical effect on the accessories and in the testosterone-treated castrate, the replacement therapy is equally effective in the normal-temperature animal and in the cold-exposed subject. Thus, in some manner, cold causes a definite depression of the endocrine activity of the testis. This depression of the accessories may be partly related to the general emaciation of the stressed subject, but somatic changes cannot affect purely endocrine organs to such an extent and induce reversal to prepubertal levels merely by sliding along the axis of body weight values. The significance of the changes in testicular cholesterol poses more problems than it answers. Variations in cholesteremia are known: seasonal in humans (36), sexual in animals. Serum cholesterol is said to be lower in the male, but the level is increased by castration, depressed by testosterone (37); in the female, the higher levels are associated with the follicular phase of the ovarian cycle (38). Gonadectomy has been shown (not without dispute) to reverse the sex differences in lipid metabolism (39); finally, some androgens have been shown to possess hypocholesteremic properties permitting species differences (40, 41).

Assuming that a significant fraction of the cholesterol present in the testes is a precursor to the testicular steroids (42,43,44), a blockage of androgen biosynthesis should cause an increase in the total precursor;

* if biosynthesis of testicular cholesterol is merely an alternate pathway to androgen formation (45), the reduced production of androgens would still result in an increase of total cholesterol. The ^{apparent} increase in total cholesterol of the testes in conjunction with the changes in the sexual indicators, confirms the fact of a severe depression of androgenic activity (46); the mechanism being a blockage of androgen production (47) or a switch to an alternate pathway. This representation is strictly provisional in the absence of data on the cholesteremia; the increase in adrenal cholesterol and sudanophilia might well point to increased local steroid synthesis (48), and delivery, but heart cholesterol as a representative of somatic events appears too erratic to lend pertinent information.

The depression of androgen production, evidenced by this disturbance of steroid biosynthesis and by the regression of the sexual accessories, indicates a clearcut depression of the endocrine activity of the testis brought about by the stress of cold; one must remember that the ages of the experimental subjects are well within the limits of full sexual activity. Worthy of note is the suspected appearance of a regeneration of the accessories in the cold-exposed castrate, presumably due to an increased titer of adrenal androgens; this effect appears on the prostate, considered a more sensitive barometer of androgenic action, but this action though minimal, seems typical enough to warrant further investigation.

CONCLUSIONS

1) Cold produces degeneration of the testicular function:

a) a depression of spermatogenesis (exocrine) associated with cryptor-

* Since the "mg of cholesterol per unit mass" expresses relationship to the whole testis, the observed change may be fictitious; indeed, the tubular degeneration may by itself account for the weight depression of the testis, resulting in a relative increase in cholesterol concentration, while the presumed source (interstitium) remains unchanged.

- chism; b) a reduction in the production of androgens (endocrine).
- 2) The degeneration observed after chronic exposure to moderate cold can be duplicated in nature and compressed in time, if cold is made more severe; the degeneration appears proportional to the intensity and duration of the stress.
- 3) Among the effects of cold, distinct areas become observable: somatic, e.g. depression of body weights; neural, e.g. reflex cryptorchism; and endocrine, e.g. depression of androgenic activity manifested by regression of the sexual indicators.
- 4) The effects of cold on the testis are not appreciably modified by age, provided the subject is sexually mature and active.
- 5) In the cold-stressed castrate, adrenal androgens are seen to act minimally but typically, after a suitable duration of stress response.
- 6) Cold "per se" has probably no topical action on the sexual accessories, since these organs retain their "indicator" value relative to the level of circulating androgens and the effectiveness of testosterone therapy.
- 7) The survival of the cold-exposed male is modified by the functional level of the endocrine testis; the response of the male appears as a physiological castration. Furthermore, exogenous testosterone is seen to lower resistance to cold by some form of toxicity. This assumption may have to be seriously revised as current observations seem to disprove the initial observations on survival of the testosterone-treated castrates.

As a working hypothesis to support further investigation, the following proposition may be stated: cold "per se" acts on the testis:

- 1) by causing reflex cryptorchism and its long-term effects on spermat-

genesis; 2) by way of somatic conditions resulting from general emaciation; 3) by way of some neuro-humoral pathway, resulting in a situation comparable with castration. The time-development of the testicular response to cold may be visualized as a definite schedule observable in the gradual responses of the accessories. Under the present conditions, no evidence was sought of a return to normal of the testicular function, endocrine or exocrine; no opinion can be expressed on the mechanism of cholesterol utilization and/or testosterone biosynthesis.

SUMMARY

The response to severe cold was studied in the mature male rat under three conditions: normal, castrate and testosterone-treated castrate. Data were obtained on 1) the alterations of the organ weights and of the histological picture of testes and adrenals, and 2) the functional changes in the sexual accessories. Physiological inferences allow us to see the effect of cold on the testis as a functional depression, exocrine and endocrine as well.

Figure 1,1.

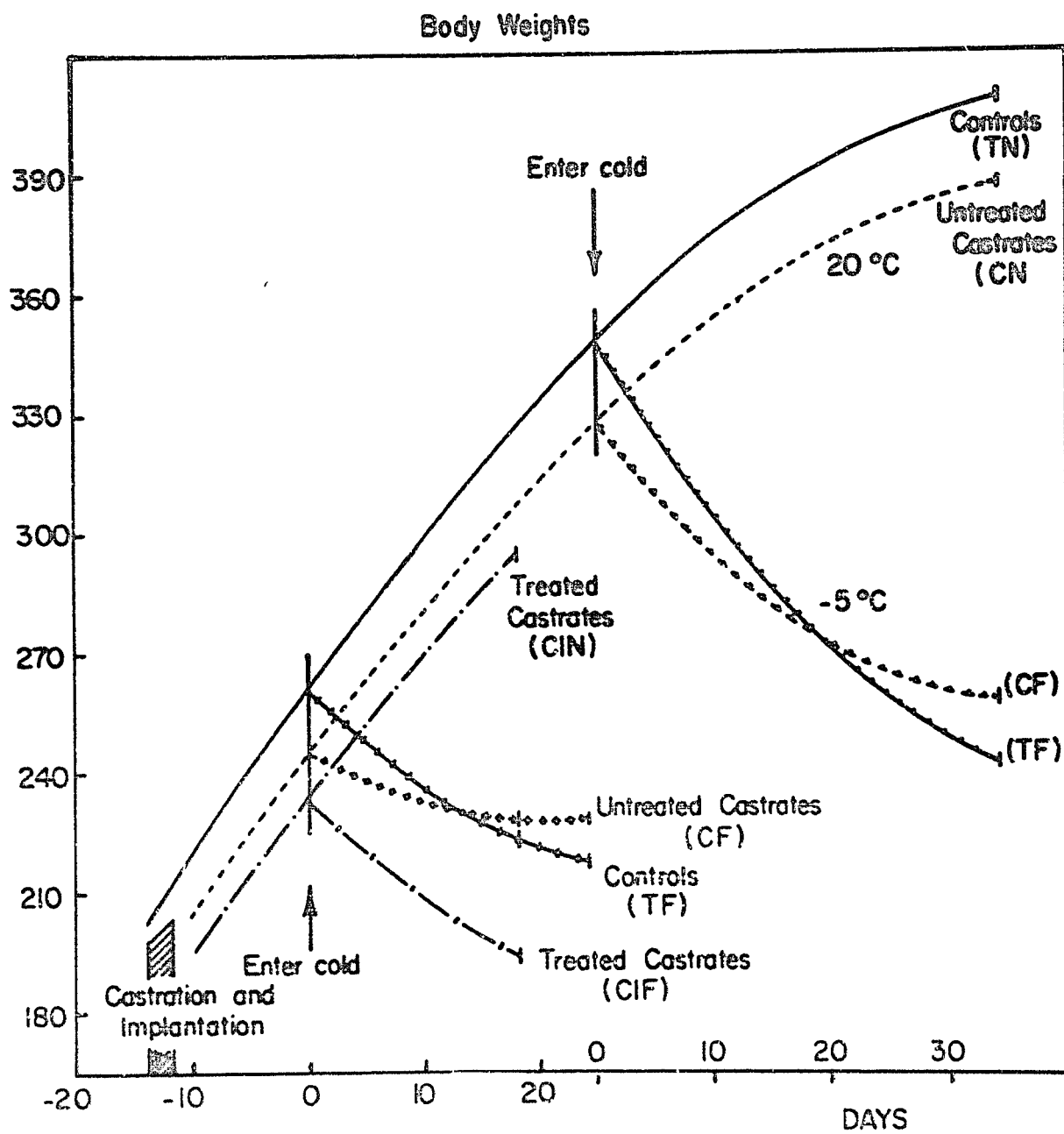


Plate I. Effect of cold on the testis of the adult rat
(125 X - Mallory trichrome).

Figure 1. 18 days - Control at room temperature (20°C).

Figure 2. 18 days - Exposed to cold (-5°C).

Figure 3. 24 days - Exposed to cold (-5°C).

Figure 4. 34 days - Exposed to cold (-5°C).

Plate 1,1.

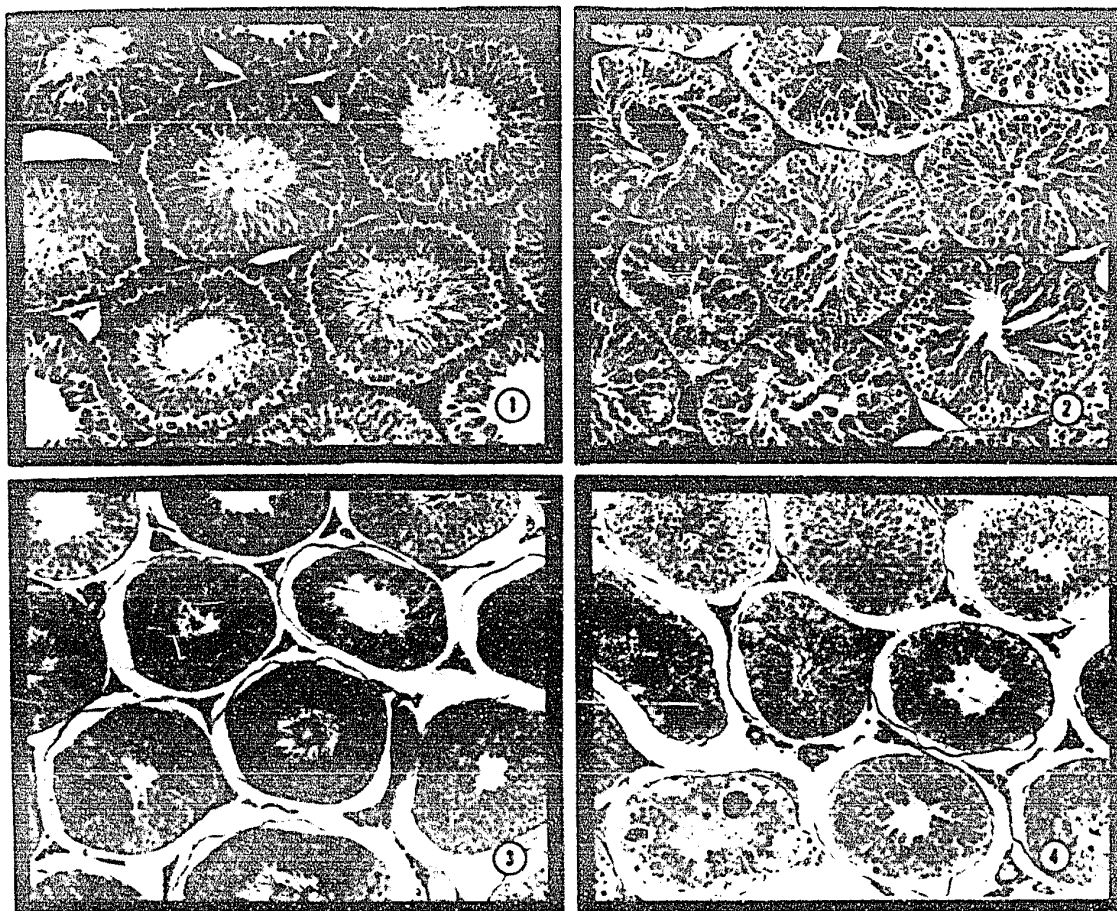


Plate II. Effect of cold on the adrenal of the adult male rat (37.5 X; figure 5 and 6: Sudan Black; figure 7-10: Mallory trichrome).

Figure 5. 18 days - Control at room temperature (20°C).

Figure 6. 18 days - Exposed to cold (-5°C).

Figure 7. 24 days - Control (20°C).

Figure 8. 24 days - Exposed to cold (-5°C).

Figure 9. 34 days - Control (20°C).

Figure 10. 34 days - Exposed to cold (-5°C).

Plate 1, II.

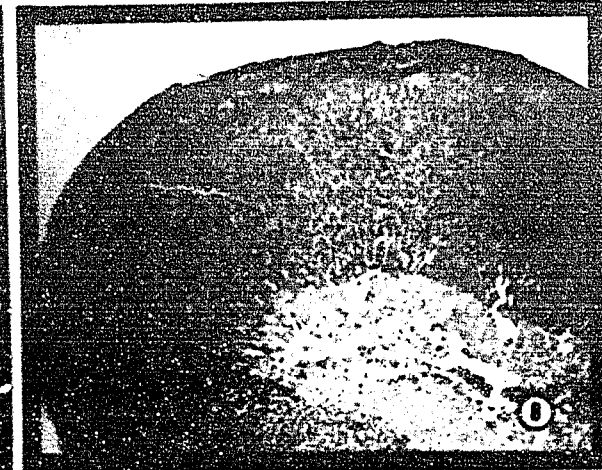
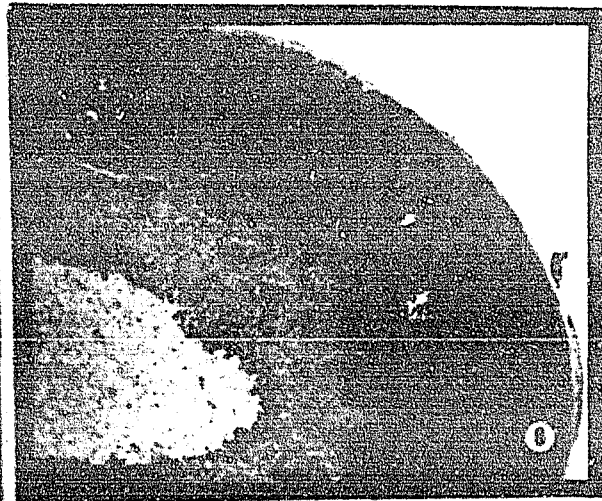
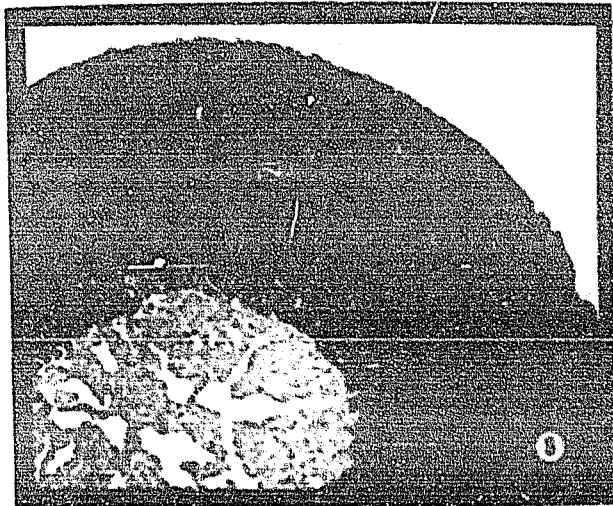


Plate III. Effect of cold, castration and testosterone on the adrenal of the adult rat (37.5 X; figure 11, 12, 15 and 16: Sudan Black; Figure 13 and 14: Mallory trichrome).

Figure 11. 18 days - Castrate at room temperature (20°C).

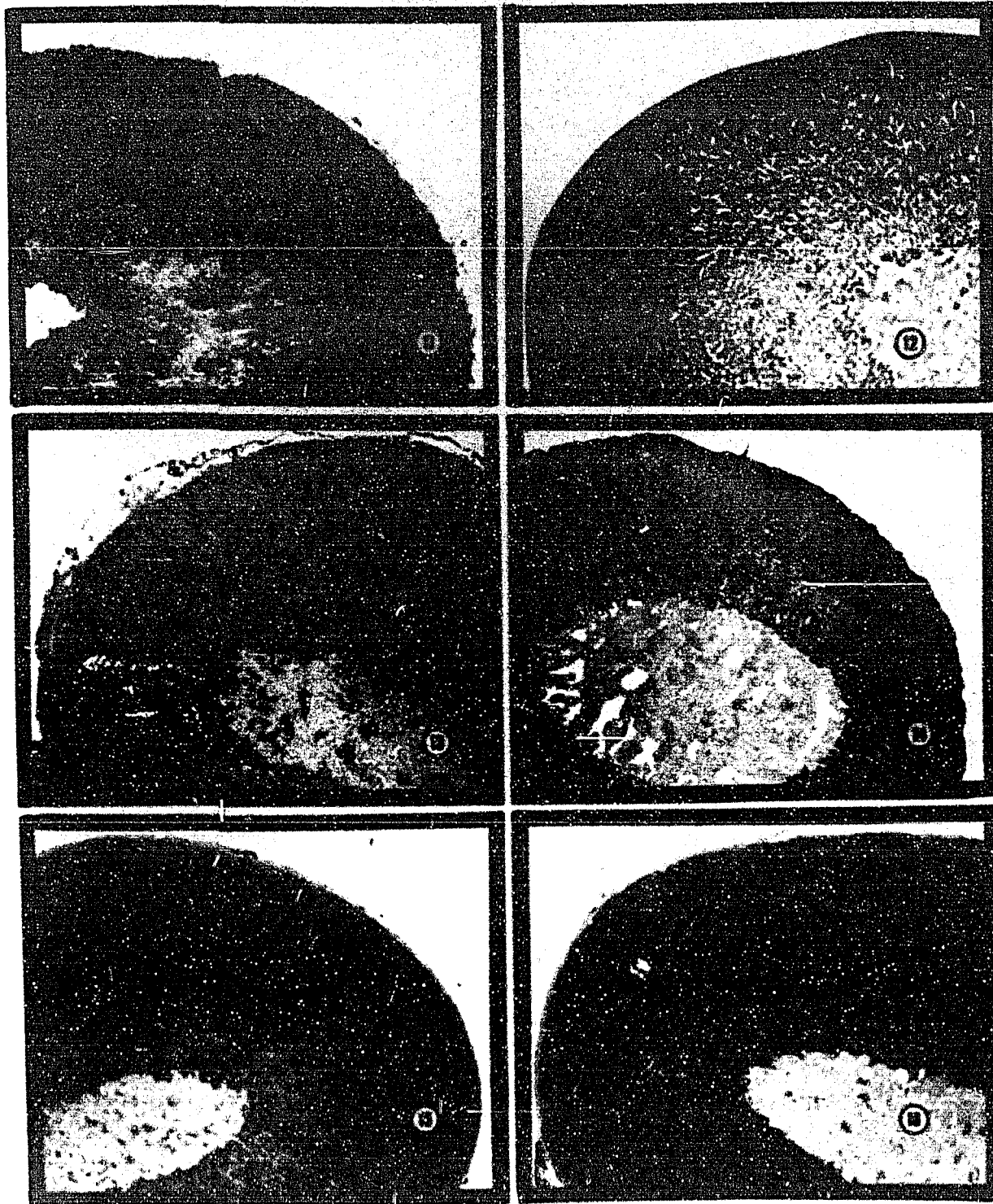
Figure 12. 18 days - Castrate exposed to cold (-5°C).

Figure 13. 24 days - Castrate at room temperature (20°C).

Figure 14. 24 days - Castrate exposed to cold (-5°C).

Figure 15. 18 days - Treated castrate at room temperature (20°C).

Figure 16. 18 days - Treated castrate exposed to cold (-5°C).



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Chapter Two

POSSIBLE PROTECTIVE ACTION OF ASCORBIC ACID
AGAINST COLD-INDUCED DAMAGE IN THE ENDOCRINE TESTIS

In the investigation of the effects of cold on the function of the testis, an examination of the action of ascorbic acid on the genital apparatus was considered imperative. Resistance and acclimatization to cold have been closely related with the tissue contents in ascorbic acid, whether complemented by an exogenous supply as in the guinea pig or increased intrinsically as in the rat (1). Thus, animals exposed to cold require greater amounts of ascorbic acid, not only for adaptation, but for maintenance and survival (2,3). Further studies revealed that the beneficial effect of ascorbic acid was possibly mediated by the thyroid hormones with the "conditioning" cooperation of the cortical steroids (4).

Nutritional studies have also demonstrated a protective action of ascorbic acid, whereby serious polyhypovitaminosis as well as specific deficiencies could be corrected or significantly prevented (5). This protective action was specially observable in the case of thiamine deficiency; in the young rat, the development of the testis is retarded, spermatogenesis is variably deferred and atrophy of the sexual accessories indicates functional impairment of the interstitial tissue. Ascorbic acid corrects partially the three areas of damage, presumably by reversing the depression of gonadotrophin production due to malnutrition (6). Scurvy in the guinea pig was demonstrated to cause in the testicular apparatus earlier and more severe damage than inanition alone (7). The total ascorbic contents of the testis was shown to be decreased in the cryptorchid testis and

in the hypophysectomized animal; however, gonadotropins inducing increased steroidogenesis did not evoke a higher level of ascorbic concentration. From these observations, the conclusion was drawn that ascorbic concentration of the testes bore no relation to steroid production and that the depression observed in the cryptorchid testes was due to changes in metabolism of the abnormally placed testis (8).

Additional evidence from histochemical and Warburg studies showed that in the scorbutic guinea pig, increased glycogen of the epithelium of the seminal vesicles was probably related to a disturbance in fructose metabolism. Total and cellular cholesterol was augmented in the testis and a decrease of succinic dehydrogenase and cytochrome oxidase activity pointed to a depression of enzymatic function in the ascorbic-deficient testes and vesicles (9). However, microscopic examination of the interstitium in scorbutic guinea pigs failed to display recognizable damage (10). Cattle studies revealed that the ascorbic acid content of bull semen was an indicator of androgenic activity, responded to HCG treatment and was unaffected by stress (undefined) (11).

In spite of these conflicting data, but in view of its action in cold acclimation, we decided to test the ability of Vitamin C as a potential protective agent against damage in the endocrine testis and against other responses observed after exposure to severe cold; if this protection is effective and reaches the anatomical level, it

should be observable in terms of improved survival, better body weight support and maintenance of the androgenic function of the testis as made visible by the response of the indicators.

MATERIALS AND METHODS

A group of male rats (Wistar Albino) was arranged according to the norm of Table I:

Table I

Distribution of animals			
Groups		20°C (N)	-5°C (F)
Controls	(T)	TN (13) ¹	TF (23)
Ascorbic acid ²	(A)	AN (20)	AF (34)

1: Group population

2: 150mg daily in 2 equal doses, by gavage needle; controls receive twice daily an equal volume (1cc) of distilled water by the same route.

After a 5-day quarantine in air-conditioned quarters, the animals were grouped and three days prior to entry into cold ascorbic acid treatment was initiated. Ascorbic acid (Redoxon Roche)* to the experimental animals and distilled water to the controls were administered by gastric gavage. The needle was fed from an automatic

* Redoxon: Vitamin C (Ascorbic acid U.S.P.) Roche; 2 cc ampulles containing 500mg of l-Ascorbic acid, graciously supplied by Hoffman-LaRoche, courtesy of Dr. Ronald M. Fyfe, Medical Director. The desired concentration (75 mg/cc) is obtained by dilution with distilled water, prepared daily and kept at refrigerator temperature.

syringe dispenser, permitting a smooth and quick operation; the animals suffered no trauma and quite early became habituated to stomach penetration by the device. Ascorbate was administered as the sodium salt of ascorbic acid; since from previous tests (with NaHCO_3 at equimolarity), the cation proved without effect, no sodium control was carried.

Exposition to cold was made in ventilated rooms at $-5^\circ\text{C} \pm 1^\circ\text{C}$ with illumination from 9:00 a.m. to 5:00 p.m. The controls were left in air-conditioned quarters maintained at approximately 20°C , under natural illumination. All animals were caged individually; Purina lab chow and tap water ad libitum for all subjects. In the cold, however, to prevent nesting, a limited but ample number of pellets was offered. Body weights were obtained every 2-3 days and daily inspection made for signs and symptoms of cold injury. On day 18, all animals were sacrificed and autopsied.

RESULTS

Body weights (Table II)

The now familiar depression due to cold is observed, at a level corresponding to an 18-day period of exposure. No effect appears due to ascorbic acid, in the terminal body weights and in the growth curves (Fig. 1).

Table II

Body weights (grams)				
Groups		Initial		Terminal
Controls	20°C	258.9 ± 2.8 ¹ (13) ²		321.8 ± 6.0 (13)
	-5°C	260.6 ± 2.6 (23)		247.0 ± 4.5 (18)
				<u>-23%</u> (31) ³
Ascorbic acid	20°C	259.4 ± 2.4 (20)		311.7 ± 5.7 (18)
	-5°C	258.5 ± 2.4 (34)		239.2 ± 3.7 (27)
				<u>-23%</u> (45)

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

 : p < 0.001

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
Initial B.W.	N.S.	N.S.	N.S.
Terminal B.W.	N.S.	t = 14.4 p < 0.001	N.S.

Survival rates (Table III).

The survival rate is similar to that previously observed and no difference due to ascorbic acid is detectable.

Table III

Survival rates (% of initial population)					
Groups	Days ₀	4	8	12	16
Controls	100 (23) ¹	96 (22)	96 (22)	82 (19)	78 (18) ²
Ascorbic acid	100 (34)	97 (33)	91 (31)	85 (29)	79 (27)

1: Initial population

2: Terminal population

Organ weights and other data

Testes (Table IV)

A divergent effect of ascorbic acid at normal temperature and in the cold proves non-significant and within the range of normal variation. The depression due to cold, at the predictable level and within the familiar range, is the only significant observation.

Table IV

		Testes (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	3.19 ± 0.07^1 (13) ²	2.99 ± 0.09 (18)	- 6% (31) ³
Ascorbic acid	(A)	3.33 ± 0.08 (19)	2.68 ± 0.09 (27)	<u>-19%</u> (46)

1, Standard error of the mean

—: $p < 0.001$

2, Group population

3, Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	Fobs.	
Effect of treatment	1	0.146	0.146	0.94	N.S.
Effect of cold	1	3.178	3.178	20.48	* *
Interaction	1	0.912	0.912	5.87	*
Error	73	11.33	0.155		

Effect of cold: $t = 5.34$ $p < 0.001$

Sexual accessories (Table V and VI)

The weight loss due to cold occurs almost exactly at the predictable point and within the familiar range; ascorbic acid proves without detectable effect.

Table V

Prostate (weight in grams)				
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	0.36 ± 0.03^1 (13) ²	0.23 ± 0.02 (18)	<u>-36%</u> (31) ³
Ascorbic acid	(A)	0.38 ± 0.02 (18)	0.21 ± 0.01 (27)	<u>-45%</u> (45)

1: Standard error of the mean

==: $p < 0.001$

2: Group population

3: Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	F obs.	
Effect of treatment	1	0.0	0.0	0.0	N.S.
Effect of cold	1	0.40	0.40	59.70	**
Interaction	1	0.007	0.007	1.06	N.S.
Error	73	0.49	0.0067		

Effect of cold $t = 7.11$ $p < 0.001$

Table VI

Seminal vesicles (weight in grams)				
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	0.54 ± 0.02 ¹ (13) ²	0.27 ± 0.02 (18)	<u>-50%</u> (31) ³
Ascorbic acid	(A)	0.52 ± 0.01 (18)	0.25 ± 0.02 (27)	<u>-52%</u> (45)

1: Standard error of the mean

==: p < 0.001

2: Group population

3: Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	Fobs.	
Effect of treatment	1	0.012	0.012	2.03	N.S.
Effect of cold	1	1.29	1.29	218.6	* *
Interaction	1	0.0	0.0	0.0	N.S.
Error	72	0.425	0.006		

Effect of cold: t = 15.3 p < 0.001

Stress indicators (Tables VII and VIII)

Cold alone causes the predictable response at a significant level: hypertrophy of the adrenals and thymolysis. No action of ascorbic acid detectable.

Table VII

Adrenals (weight in milligrams)				
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	45.8 ± 0.9 ¹ (13) ²	56.7 ± 1.7 (18)	+19% (31) ³
Ascorbic acid	(A)	48.4 ± 1.7 (19)	57.4 ± 1.5 (26)	+16% (45)

- 1: Standard error of the mean : p < 0.001
 2: Group population
 3: Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	Fobs.	
Effect of treatment	1	51.6	51.6	1.07	N.S.
Effect of cold	1	7191.6	7191.6	148.9	* *
Interaction	1	16.2	16.2	0.34	N.S.
Error	72	3477.8	48.3		

Effect of cold: t = 6.28 p < 0.001

Table VIII

Thymus (weight in grams)				
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	0.44 ± 0.03^1 (13) ²	0.20 ± 0.01 (17)	<u>-55%</u> (30) ³
Ascorbic acid	(A)	0.42 ± 0.02 (19)	0.18 ± 0.02 (25)	<u>-57%</u> (44)

1: Standard error of the mean

==: $p < 0.001$

2: Group population

3: Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	Fobs.	
Effect of treatment	1	0.0072	0.0072	1.08	N.S.
Effect of cold	1	1.01	1.01	15.1	**
Interaction	1	0.0	0.0	0.0	N.S.
Error	70	0.467	0.0067		

Effect of cold: $t = 12.0$ $p < 0.001$

Pituitary (Table IX)

Cold causes the predictable weight loss. A low grade of significance marks the effect of ascorbic at room temperature; a similar effect was occasionally seen after short treatment periods, but no relationship with time could be established.

Table IX

Pituitary (weight in milligrams)				
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	9.0 ± 0.4 ¹ (13) ²	8.4 ± 0.4 (18)	- 7% (31) ³
Ascorbic acid	(A)	10.4 ± 0.3 (19)	8.3 ± 0.2 (26)	<u>-20%</u> (45)

1: Standard error of the mean

—: p < 0.001

2: Group population

3: Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	Fobs.	
Effect of treatment	1	9.04	9.04	4.11	*
Effect of cold	1	30.6	30.6	13.9	**
Interaction	1	10.2	10.2	4.6	*
Error	72	158.2	2.2		

Effect of treatment: t = 2.05 p < 0.05

Effect of cold : t = 3.61 p < 0.001

Liver (Table X)

No change due to cold or ascorbic acid or both is detectable.

Table X

Liver (weight in grams)				
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	14.3 ± 0.6 ¹ (13) ²	13.3 ± 0.3 (18)	-7%(31) ³
Ascorbic acid	(A)	13.8 ± 0.3 (16)	13.8 ± 0.3 (27)	0%(43)

1: Standard error of the mean

2: Group population

3: Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	Fobs.	
Effect of treatment	1	0.16	0.16	0.06	N.S.
Effect of cold	1	2.64	2.64	1.05	N.S.
Interaction	1	4.31	4.31	1.71	N.S.
Error	70	176.6	2.52		

Lungs (Table XI)

The depression due to cold already noted is observed, but ascorbic acid is without effect.

Table XI

Lungs (weight in grams)				
Groups		20°C (N)	-5°C (F)	Difference
Controls	(T)	1.32 ± 0.02^1 (13) ²	1.16 ± 0.03 (15)	<u>-12%</u> (28) ³
Ascorbic acid	(A)	1.32 ± 0.03 (18)	1.14 ± 0.02 (26)	<u>-11%</u> (44)

1: Standard error of the mean

—: $p < 0.001$

2: Group population

3: Aggregate population

Analysis of variance

Source of variance	D.F.	S.S.	M.S.	Fobs.	
Effect of treatment	1	0.0	0.0	0.0	N.S.
Effect of cold	1	0.48	0.48	40.34	* *
Interaction	1	0.002	0.002	0.14	N.S.
Error	68	0.81	0.012		

Effect of cold: $t = 6.8$ $p < 0.001$ Water contents (Table XII)

As already noted, no change in water contents is observed consequent to cold; the only change due to ascorbic acid is a non-significant increase of 7% water contents in the adrenal at room temperature, perhaps resulting from moderate storage of exogenous vitamin C.

Table XII

% Water contents					
Groups	Testes	Prostate	Seminal vesicles	Adrenals	Thymus
Controls					
20°C (TN)	86.9 ± 0.1 ¹ (13) ²	80.9 ± 1.0 (12)	77.0 ± 0.4 (13)	68.6 ± 1.3 (13)	77.8 ± 0.8 (12)
-5°C (TF)	86.2 ± 0.2 (18)	78.7 ± 1.2 (18)	76.6 ± 0.9 (18)	72.4 ± 0.8 (17)	76.3 ± 1.4 (18)
Ascorbic acid					
20°C (AN)	87.0 ± 0.1 (19)	81.5 ± 0.9 (19)	77.0 ± 0.3 (17)	73.6 ± 0.4 (18)	77.2 ± 0.9 (18)
-5°C (AF)	86.0 ± 0.4 (27)	78.7 ± 1.2 (27)	77.4 ± 0.4 (24)	72.8 ± 0.5 (26)	76.0 ± 0.8 (25)

1: Standard error of the mean

2: Group population

Analysis of variance - summary				
Testes	Prostate	Vesicles	Adrenals	Thymus
Effect of treatment	N.S.	N.S.	N.S.	N.S.
Effect of cold	N.S.	N.S.	N.S.	N.S.
Interaction	N.S.	N.S.	N.S.	N.S.

Microscopic observations

The familiar picture of spermatogenic damage due to cold was observed along with the debatable observations on the interstitial spaces. (Plate I, Figs. 2 and 6). No evidence of difference or repair due to vitamin C was observed. (Plate I, Figs. 4 and 8).

DISCUSSION

Effect of cold

The present observations confirm previous data on the effect of cold and fall almost exactly at the values predictable for the duration of the exposure; the pattern of response in the various data is now familiar and requires no further comment. The survival in the cold remains unaffected by ascorbic treatment.

Effect of Vitamin C

At room temperature, the only observation worthy of note is an unexplained elevation of pituitary weight in the ascorbic-treated subjects.

All the observations on the cold-exposed animals proved negative for any type of influence due to ascorbic acid. The dosage was selected to provide an excess of circulating Vitamin C; indeed, this dose level (150 mg/day/rat) was adopted from previous observations where 325 mg/day had proven effective in monkeys with a body weight of 5.6 kg exposed to moderate cold (3).

On the basis of the presumed participation to progesterone synthesis (12) and action on steroidogenesis (13), we had hoped to detect some effect in the sex indicators. Adrenal hypertrophy (sign of non-adaptation) shows the inability of ascorbic acid to protect against cold under the present conditions.

Speculating on the negative results, one could perhaps say that the stress of intense cold acts with such acuity and swiftness as to prevent any form of adaptation; thus, partial protection if present, against biochemical injury, would be overshadowed and would never reach the anatomical level, to render protection and repair observable.

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Figure 2, 1.

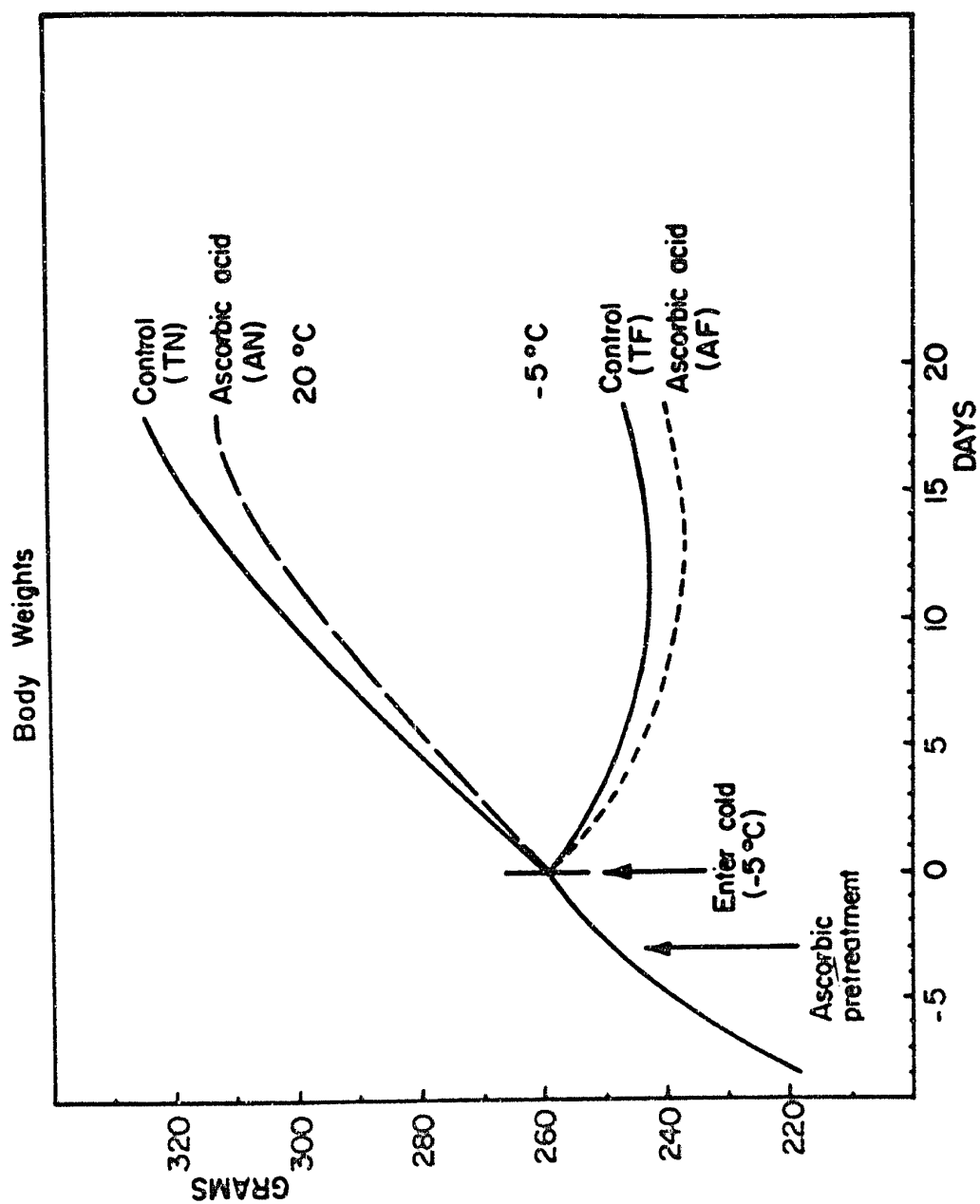
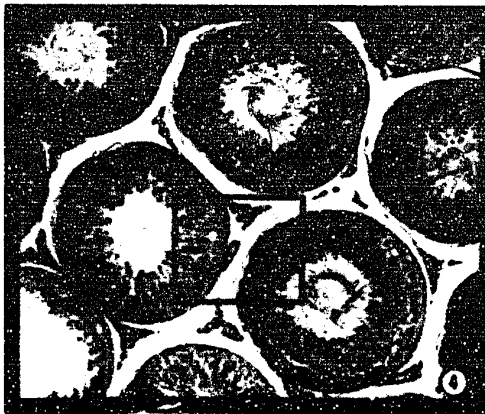
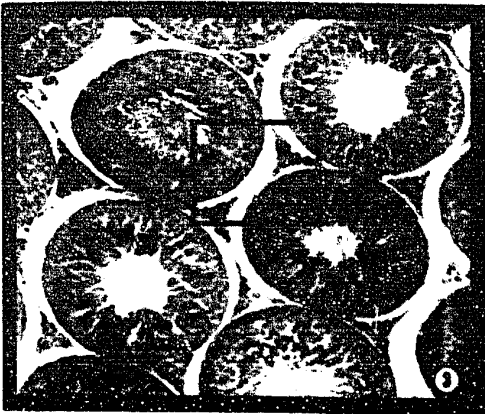
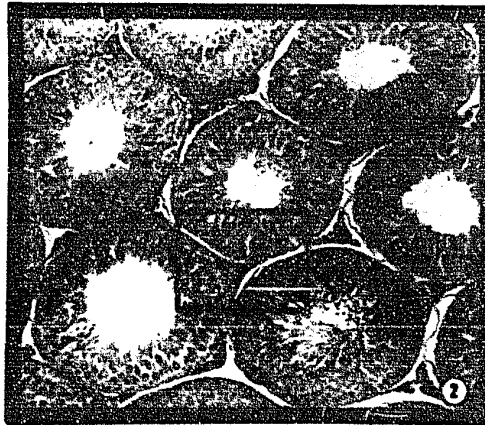
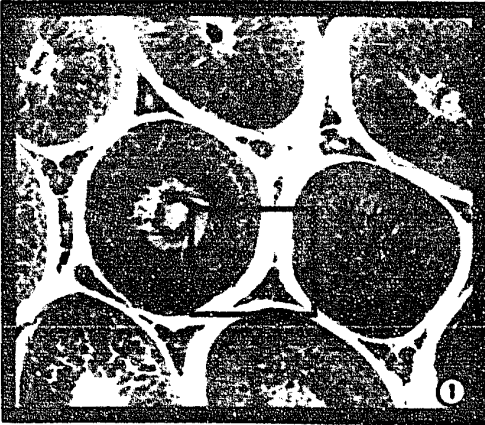


Plate I. Effect of ascorbic acid and cold on the testis
of the adult rat (Mallory trichrome).

125 X

500 X

- Figure 1. Figure 5. Control at room temperature (20°C).
Figure 2. Figure 6. Exposed to cold (-5°C).
Figure 3. Figure 7. Ascorbic acid at room temperature (20°C).
Figure 4. Figure 8. Ascorbic acid exposed to cold (-5°C).



Chapter Three

POSSIBLE PROTECTIVE ACTION OF ZINC AGAINST
COLD-INDUCED DAMAGE IN THE ENDOCRINE TESTIS

Since Bertrand and Vladesco first demonstrated the presence and discussed the function of zinc in the male reproductive organs (1), numerous investigations followed the premise that "zinc plays a part in reproduction and in the regulation of internal secretions".

Moderate zinc deficiency obtained by restriction of the dietary intake was shown to cause degeneration of the testes and of the sexual accessories; however, retarded accessories responded to testosterone and the testes recovered under gonadotropin treatment, leading to the assumption that zinc favors production of these hormones (2). The systemic effects of a dietary absence of zinc were corrected by supplement, except the atrophy of the testes and the degeneration of the accessories (3). Attendant to the various levels of zinc deficiency, corresponding changes were observed in the zinc contents of the dorso-lateral prostate. An excess of topical zinc caused by intratesticular injection of zinc chloride induced teratomas in the rooster, if the subject was treated during the spring period of activity and especially after gonadotropin "priming"; in the rat, where spontaneous tumors of the genital tract are exceptional, testicular growths were obtained, but only after an extremely long period (at least 15 months) (4).

A high concentration of zinc was demonstrated in the dorsolateral prostate of the rat (and in the Paneth cells of the rat small intestine), with only a small fraction (2.5%) in carbonic anhydrase (5). The increase in prostatic zinc progresses through infancy to a maximum of 160 days

of age; this increase parallels carbonic anhydrase contents, but is unrelated to carbonic anhydrase or phosphatase (acid or alkaline) activities (6).

Removal of zinc from the semen did not seem to affect fertility, showing that zinc may be important but not essential to reproduction (7); although spermatozoa contain a high concentration of zinc (8) presumably extracted from the seminiferous tubules, some authors leave open the question of the function of zinc (9). In human semen, zinc is said to be present in 2 states: 1) as free ions and 2) as protein-bound zinc deriving from prostatic fluid; no association of zinc with seminal lipids is known (10).

In vitro studies of Zn^{65} uptake by the prostate have been used in the evaluation of prostatic diseases (11); in the hypophysectomized rat, the same response has been considered a sensitive and specific bioassay for ICSH activity (12). The uptake varied with the different regions of the gland and displayed modifications attendant to cancerous conditions (13). The testicular uptake of Zn^{65} appeared depressed 18 days after hypophysectomy, although changes in the germinal epithelium and in the interstitium were still minimal; ICSH given on the 5th post-operative day prevented depression of the uptake, but STH and prolactin proved ineffective (14). Testosterone in the zinc-deficient rat, improved growth of the accessories without improving the concentrating power and without correcting testicular atrophy; gonadotropins favored the rate of growth and the sexual maturation in the young, but did not reverse the genital atrophy (15). ACTH in the hypophysectomized rat restored

the uptake ability of the prostate and raised the zinc concentration in the prostate and in the adrenals; thus, the possibility of an influence of zinc on steroidogenesis was accepted (16). Adrenalectomy depressed the Zn^{65} concentration and the uptake ability of the testis and of the dorsolateral prostate; cortisone returned this property to normal (17).

Other trace elements pose interesting problems to the endocrinologist; serum copper has been shown to increase significantly in elderly males and in post-menopausal females under testosterone and estradiol respectively; 8 weeks after cessation of either treatment, the serum contents had returned to the pre-treatment level (18). A more dramatic case in point is cadmium; this element, more toxic to the male than to the female, administered in non-toxic levels caused acute destruction of the germinal epithelium and of the interstitial tissue, evoking castration phenomena (19). Twenty days after subcutaneous injection, the Leydig cells began to regenerate and the endocrine function returned gradually, but restoration of the germinal epithelium was delayed to at least 133 days. A large dose of zinc salts (also by subcutaneous injection) prevented this spectacular damage, presumably by inhibiting the cadmium blockage of the SH groups; this antagonism had already been recognized in the case of yeast growth (20).

The cadmium damage is not species specific, but is restricted to the testis; Zn^{65} uptake is depressed in the testis and in the dorsolateral prostate, but not in the ventral prostate nor in the ovary or

other tissues of the female genital tract. The conclusion is drawn that the zinc complexes in the testis and dorsolateral prostate (under ICSH and androgen control) might be different from the zinc complexes in other organs (21). Thus, zinc would be displaced by cadmium for which no homeostatic control seems to exist, whereas zinc appears to be subject to two different controls: 1) a negative feedback towards absorption and 2) a positive feedback towards excretion (22). The cadmium damage to the testicular function has ingeniously been utilized for the localization in the pituitary of specific gonadotrophs, some acting on the germinal epithelium, others acting on the interstitium (23).

In view of the importance of zinc in the physiology of the testis and in relation with findings on the effect of cold in the male genital apparatus, it was natural to think of a supporting or protective action of zinc towards the testis under stress. The protection, if present, could appear at the somatic level by improvement of survival and better maintenance of the body weight and growth process, and/or at the physiological level, by anatomically observable prevention of damage to spermatogenesis or androgenic activity.

MATERIALS AND METHODS

A group of male rats (Wistar Albino) was arranged according to the norm of Table I. All procedures were not performed simultaneously nor on homogeneous groups. However, the data were pooled only when statistical evaluation showed acceptable replication.

Table I

Distribution of animals			
Groups		20°C (N)	-5°C (F)
Controls	(T)	TN (73) ¹	TF (220)
Zinc-treated	(Z) ²	ZN (88)	ZF (210)

1: Group population

2: 300 micrograms of $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ (0.3 ml) daily at same hour by gavage needle; controls receive distilled water under same conditions.

The zinc treatment was initiated upon entry into cold and proceeded as follows: a solution of $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ (1mg/ml) was administered daily at the same hour by gavage needle at the dosage of approximately 300 μgm (0.3 ml). This dosage was selected because of its proven replacement value in zinc-deficient animals. The manipulation proved non traumatic, quick and easy, as the animals became promptly habituated to the insertion of the device. This method of direct administration was preferred to "ad libitum" drinking, for a number of reasons: at -5°C, the drinking water freezes requiring frequent changes of containers and although the animals eventually learn "ice chewing", the amount of fluid consumed by individual subjects remains highly variable; furthermore, the cold-exposed animals consume less water than the subjects at room temperature. For these reasons, a uniform technique was adopted.

Distilled water was administered to the controls by the same method.

RESULTS

Body weights (Table II - Terminal body weights)

The familiar pattern of growth arrest and weight loss is observable, where the depression is related to duration of exposure; no difference appears as a consequence of zinc treatment. The growth curves of controls versus zinc-treated are practically coincident and display no significant effect except the action of cold. Thus, figure 3,1 displays one single curve for the subjects at room temperature (with or without Zinc) and one single curve for the cold-exposed animals (with or without Zinc treatment).

Table II

Body weights (grams)				
Groups		Duration of exposure (-5°C)		
Initial		17 days	30 days	40 days
Controls	(20°C)	264.7 ± 2.7 ¹ (35) ²	272.9 ± 2.8 (23)	264.0 ± 3.2 (15)
	(-5°C)	265.3 ± 1.5 (93)	269.3 ± 2.3 (67)	266.1 ± 2.4 (60)
Zinc	(20°C)	265.0 ± 2.2 (39)	265.9 ± 1.4 (25)	265.2 ± 1.8 (16)
	(-5°C)	265.5 ± 1.4 (93)	264.2 ± 1.8 (58)	266.1 ± 2.0 (59)
Terminal				
Controls	(20°C)	333.3 ± 3.5 (23)	441.9 ± 0.7 (13)	372.5 ± 17.8 (6)
	(-5°C)	225.3 ± 2.4 (42) <u>-32%</u> (65) ³	223.4 ± 2.3 (64) <u>-50%</u> (77)	218.0 ± 9.4 (9) <u>-41%</u> (15)
Zinc	(20°C)	325.3 ± 2.7 (28)	424.7 ± 3.2 (10)	365.8 ± 23.5 (6)
	(-5°C)	233.1 ± 3.3 (40) <u>-28%</u> (68)	216.6 ± 2.8 (56) <u>-49%</u> (66)	224.2 ± 8.4 (11) <u>-39%</u> (17)
		(April 25 to May 12 and Nov. 4 to Nov. 20)	(Aug. 9 to Sept. 18)	(Nov. 4 to Dec. 12)

- 1: Standard error of the mean : p < 0.001
 2: Group population
 3: Aggregate population

Analysis of variance - summary

Initial Body Weights

Effect of treatment	N.S.	N.S.	N.S.
Effect of cold	N.S.	N.S.	N.S.
Interaction	N.S.	N.S.	N.S.

Terminal Body Weights

Effect of treatment	N.S.	N.S.	N.S.
Effect of cold	t = 32.9 p < 0.001	t = 34.5 p < 0.001	t = 8.9 p < 0.001
Interaction	*	N.S.	N.S.

Survival rates (Table III).

The pattern of the survival rates displays two familiar features:

- 1) the rate of death is swift at first, then tapers off as the more resistant subjects survive and exceptionally, may be considered adapted;
- 2) the survival at 18 days is at the level previously observed which points to a pleasant uniformity of response.

Table III

		Survival rates (% of initial population)								
		Days								
		0	10	20	30	40	50	60	70	80
Controls	100	73	45	45	35	26	16	13	7	
Zinc	100	76	48	42	25	18	18	13	8	

Organ weights and other data.

Testes weight (Table IV)

The predictable loss of organ weight and the time effect are clearly observable within the usual range; the only significant effect is due to cold, no action of the zinc treatment detectable.

Table IV

		Testes (weight in grams)		
Groups		Duration of exposure (-5°C)		
		17 days	30 days	40 days
Controls	(20°C)	3.42 ± 0.06 ¹ (24) ²	3.18 ± 0.08 (13)	3.22 ± 0.05 (5)
	(-5°C)	2.68 ± 0.04 (41)	2.40 ± 0.11 (21)	1.90 ± 0.20 (9)
		<u>-22%</u> (65) ³	<u>-25%</u> (34)	<u>-41%</u> (14)
Zinc	(20°C)	3.17 ± 0.05 (28)	3.06 ± 0.06 (9)	3.32 ± 0.10 (6)
	(-5°C)	2.71 ± 0.06 (38)	2.13 ± 0.13 (20)	2.33 ± 0.20 (11)
		<u>-15%</u> (66)	<u>-30%</u> (29)	<u>-30%</u> (17)

1: Standard error of the mean

—: p < 0.01

2: Group population

3: Aggregate population

—: p < 0.001

Analysis of variance - summary

Effect of treatment	N.S.	N.S.	N.S.
Effect of cold	t = 10.4 p < 0.001	t = 8.6 p < 0.001	t = 6.3 p < 0.001
Interaction	*	N.S.	N.S.

Total testis cholesterol (Table V)

A check of this variable confirmed several previous observations, and shows the effect of cold as the only significant cause of alteration.

Table V

Total testis cholesterol (mg % gram wet tissue)			
Groups	Duration of exposure (-5°C)		
	17 days		30 days
Controls (20°C)	152.6 ± 3.5 ¹	(13) ²	146 ± 9 (8)
	(-5°C)	183.3 ± 7.6 (26)	219 ± 6 (6)
		+17% (39) ³	+33% (14)
Zinc (20°C)	162.1 ± 8.0	(18)	163 ± 6 (9)
	(-5°C)	173.3 ± 4.1 (23)	251 ± 37 (5)
		+6% (41)	+35% (14)

- 1: Standard error of the mean — —: p < 0.05
 2: Group population — —: p < 0.01
 3: Aggregate population — —: p < 0.001

Analysis of variance - summary

Effect of treatment	N.S.	N.S.
Effect of cold	t = 3.11 p < 0.01	t = 3.42 p < 0.01
Interaction	N.S.	N.S.

Sex accessories (Tables VI and VII).

The now familiar action of cold, absolutely unaffected by zinc treatment, appears related to duration of exposure and the changes fall within the predictable range.

Table VI

		Prostate (weight in grams)		
		Duration of exposure (-5°C)		
Groups		17 days	30 days	40 days
Controls	(20°C)	0.56 ± 0.02 ¹ (24) ²	0.76 ± 0.06 (13)	0.64 ± 0.05 (6)
	(-5°C)	0.24 ± 0.01 (42)	0.19 ± 0.02 (22)	0.21 ± 0.03 (9)
		<u>-57%</u> (66) ³	<u>-75%</u> (35)	<u>-67%</u> (15)
Zinc	(20°C)	0.51 ± 0.02 (27)	0.72 ± 0.04 (10)	0.70 ± 0.01 (5)
	(-5°C)	0.24 ± 0.01 (40)	0.17 ± 0.01 (22)	0.25 ± 0.02 (10)
		<u>-53%</u> (67)	<u>-76%</u> (32)	<u>-64%</u> (15)

1: Standard error of the mean

2: Group population

3: Aggregate population

==: p < 0.001

Analysis of variance - summary

Effect of treatment	N.S.	N.S.	N.S.
Effect of cold	t = 7.25 p < 0.001	t = 15.6 p < 0.001	t = 12.6 p < 0.001
Interaction	N.S.	N.S.	N.S.

Table VII

		Seminal vesicles (weight in grams)		
		Duration of exposure (-5°C)		
Groups		17 days	30 days	40 days
Control	(20°C)	0.57 ± 0.02 ¹ (23) ²	0.66 ± 0.02 (13)	0.62 ± 0.04 (6)
	(-5°C)	0.26 ± 0.01 (41)	0.29 ± 0.02 (22)	0.24 ± 0.03 (9)
		<u>-51%</u> (64) ³	<u>-56%</u> (35)	<u>-61%</u> (15)
Zinc	(20°C)	0.55 ± 0.02 (27)	0.61 ± 0.04 (10)	0.73 ± 0.02 (5)
	(-5°C)	0.22 ± 0.01 (39)	0.21 ± 0.02 (22)	0.29 ± 0.02 (11)
		<u>-60%</u> (66)	<u>-66%</u> (32)	<u>-60%</u> (16)

1: Standard error of the mean

 : p < 0.001

2: Group population

3: Aggregate population

Analysis of variance - summary

Effect of treatment	N.S.	N.S.	N.S.
Effect of cold	t = 19.4 p < 0.001	t = 13.9 p < 0.001	t = 11.1 p < 0.001
Interaction	N.S.	N.S.	N.S.

Stress indicators (Tables VIII, IX).

No effect whatever due to zinc-treatment can be observed at room temperature or in the cold; cold alone and at the predictable level is the cause of a significant alteration. A check on total adrenal cholesterol displayed a broad variation without detectable significance, except perhaps a trend toward depression due to cold.

Table VIII

Adrenal glands (weight in milligrams)				
Groups	Duration of exposure (-5°C)			
	17 days	30 days	40 days	
Controls (20°C)	44.5 ± 1.6 ¹ (24) ²	42.4 ± 2.0 (12)	48.0 ± 4.0 (6)	
	(-5°C) 55.7 ± 1.5 (40)	49.8 ± 2.5 (22)	65.2 ± 4.2 (9)	
	+20% (64) ³	+15% (34)	+26% (15)	
Zinc (20°C)	45.4 ± 1.1 (28)	40.9 ± 1.6 (9)	51.5 ± 4.7 (6)	
	(-5°C) 58.4 ± 1.3 (40)	51.2 ± 1.7 (22)	59.5 ± 3.5 (10)	
	+22% (68)	+20% (31)	+14% (16)	

1: Standard error of the mean

2: Group population

3: Aggregate population

—: p < 0.05

—: p < 0.01

—: p < 0.001

Analysis of variance - summary

Effect of treatment	N.S.	N.S.	N.S.
Effect of cold	t = 3.67 p < 0.01	t = 4.44 p < 0.001	t = 2.95 p < 0.01
Interaction	N.S.	N.S.	N.S.

Table IX

Groups	Duration of exposure (-5°C)		
	17 days		30 days
	Thymus (weight in grams)	Pituitary (weight in milligrams)	Adrenal cholesterol (mg % gm wet tissue)
Controls (20°C)	0.44 ± 0.02^1 (15) ²	9.8 ± 0.2 (13)	3284 ± 223 (8)
	(-5°C) 0.13 ± 0.01 (27)	8.6 ± 0.2 (26)	2877 ± 400 (3)
	<u>-70%</u> (42) ³	<u>-12%</u> (39)	<u>-12%</u> (11)
Zinc	(20°C) 0.38 ± 0.01 (18)	10.1 ± 0.2 (18)	3023 ± 387 (5)
	(-5°C) 0.12 ± 0.01 (24)	8.6 ± 0.2 (24)	2195 (1)
	<u>-68%</u> (42)	<u>-15%</u> (42)	<u>-28%</u> (6)

1: Standard error of the mean

—: $p < 0.001$

2: Group population

3: Aggregate population

Analysis of variance - summary

	Thymus	Pituitary	Adrenal cholesterol
Effect of treatment	N.S.	N.S.	N.S.
Effect of cold	$t = 20.7$ $p < 0.001$	$t = 7.37$ $p < 0.001$	N.S.
Interaction	*	N.S.	N.S.

MICROSCOPIC OBSERVATIONS

1. Effect of cold and time

The now familiar pictures of the effects of cold on the spermatogenesis and on the interstitium appear clearly; the duration of the cold stress is shown in the progressive damage detectable between the shorter and the longer exposure periods.

Attention is called to Figures 10 and 14, of an untreated cold-exposed subject (25 days); the damage appears significantly greater than in a comparable subject after 50 days (Figures 18 and 22). In effect, the extent of spermatogenic and interstitial damage admits a wide range of variation; furthermore, the photomicrographs submitted were made on specimens chosen at random and the exact fields shown were selected because of their photogenicity rather than for their intrinsic features; this, we felt safe in obtaining an unbiased group of representatives.

2) Effect of Zinc

Neither at room temperature, nor in the cold, does any detectable sign distinguish the zinc-treated from the control subjects. These images are strictly histological and convey no histochemical information; nevertheless, any action of zinc, if present, fails to reach the observable anatomical level.

DISCUSSION

Action of cold

No comment is offered other than the following statement: the stress response, general and testicular, occurs exactly in the form and at the level predictable from the previous observations. Histological examination of the testes confirmed previous observations of a severe damage grossly related to the duration of exposure, but impossible to correlate with specific endocrine changes; thus, the sex accessories remain the only reliable barometer of androgenic activity.

Action of zinc

No protection effect due to zinc treatment is anatomically detectable, macroscopically or histologically. The systemic results of cold exposure, body weight loss, stress responses, display no action due to zinc treatment. The damage to the germinal sequence is equally severe and the regression of the accessories confirms equal damage to the androgenic function of the testis. A time check, at days 4 and 8, failed to reveal any difference from the predictable course of events (24).

Thus, irrespective of possible metabolic events or biochemical compensations, no effect due to zinc reaches the anatomical level; perhaps, the massive disturbance due to severe cold cancels out normal adjustments and from the start, all homeostatic mechanisms break down under the onslaught of the extremely severe stress. It is not surprising therefore, that adaptation to severe cold under the present conditions, has proved almost impossible.

Figure 3 , 1 .

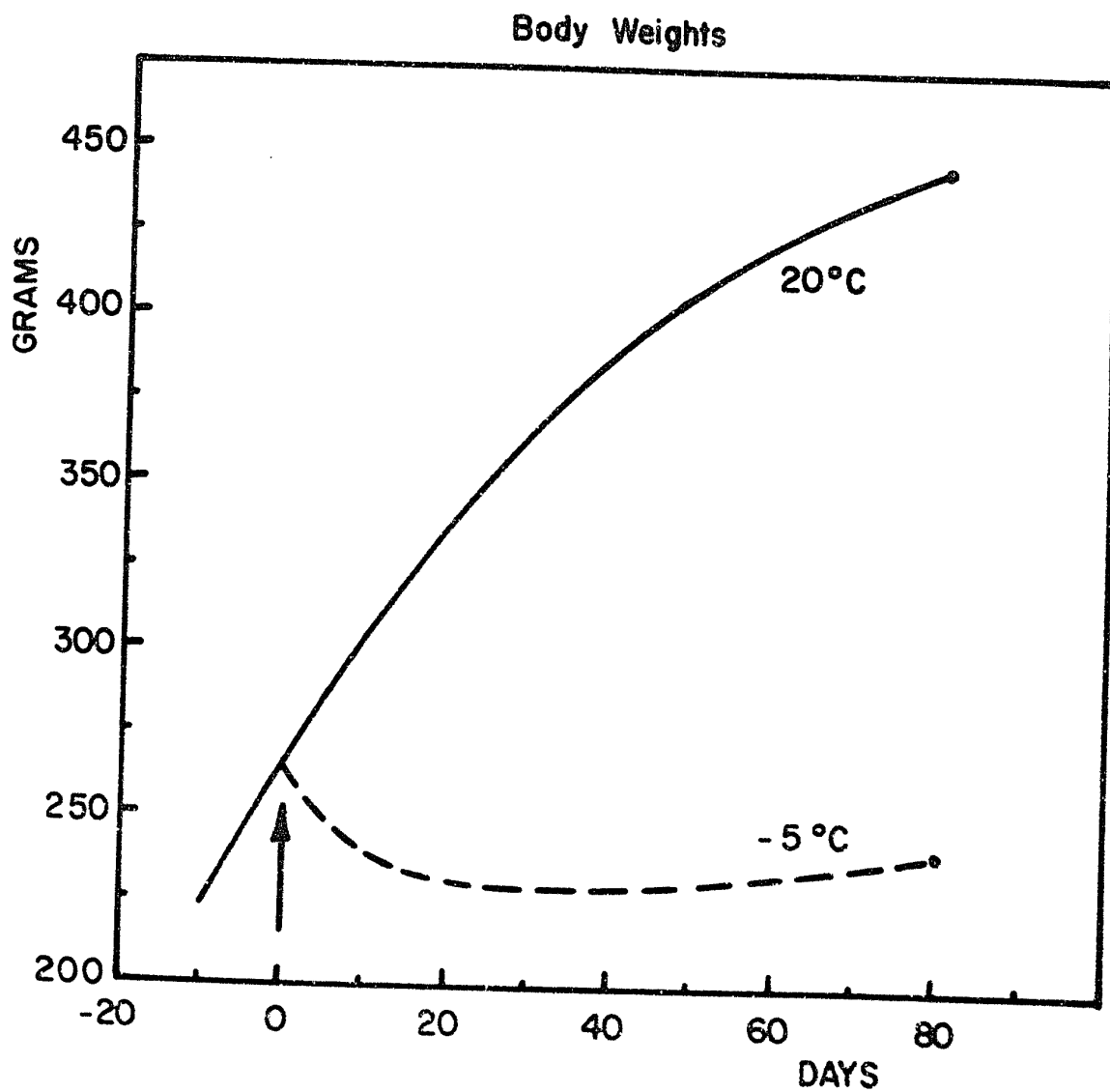


Plate I. Effect of oral Zinc and cold on the testis
of the adult rat: duration of exposure:
17 days (Mallory trichrome).

125 X

500 X

- | | |
|-----------|---|
| Figure 1. | Figure 5. Control at room temperature (20°C). |
| Figure 2. | Figure 6. Exposed to cold (-5°C). |
| Figure 3. | Figure 7. Oral Zinc at room temperature (20°C). |
| Figure 4. | Figure 8. Oral Zinc exposed to cold (-5°C). |

Plate 3, I.

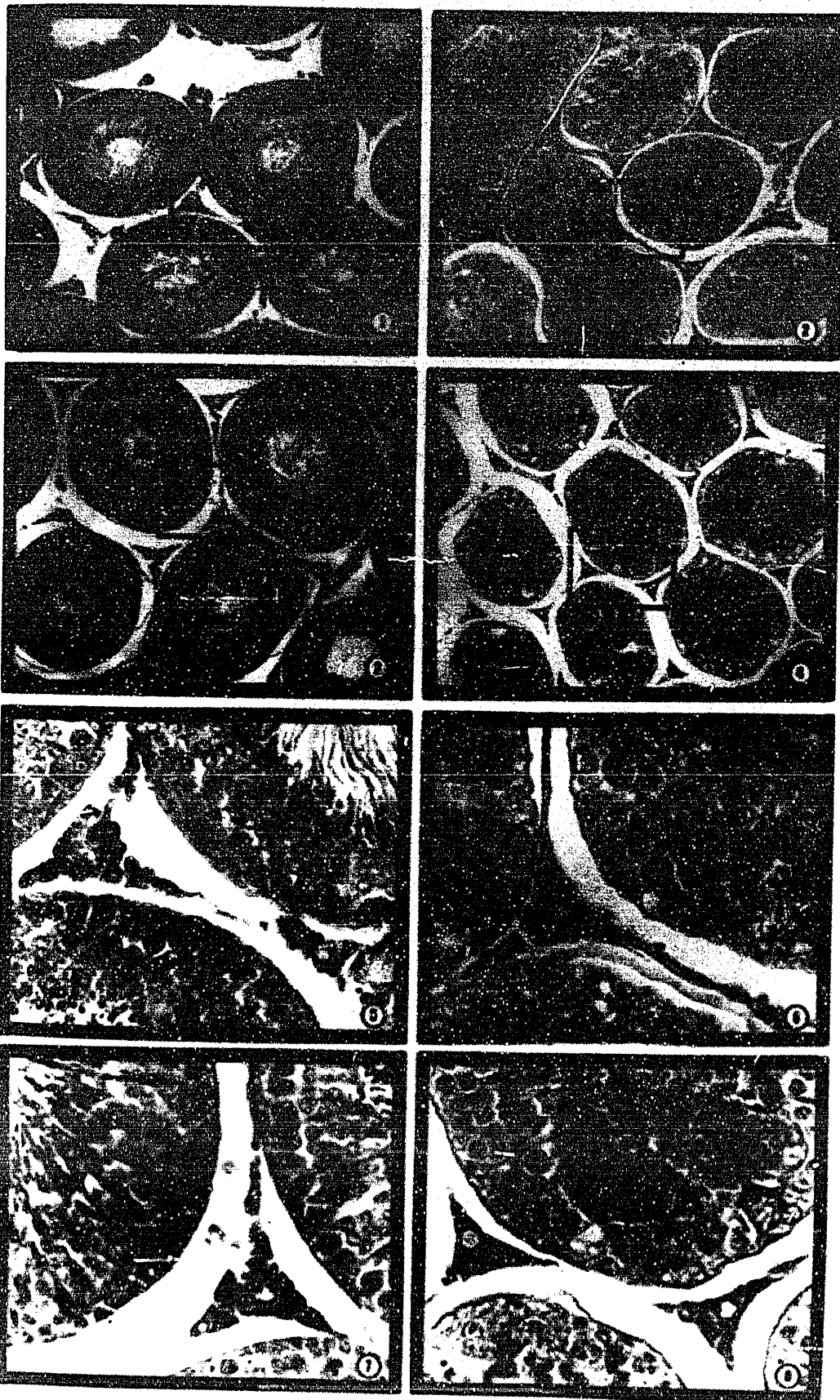


Plate II. Effect of oral Zinc and cold on the testis
of the adult rat; duration of exposure:
25 days (Mallory trichrome).

125 X

500 X

- | | |
|------------|--|
| Figure 9. | Figure 13. Control at room temperature (20°C). |
| Figure 10. | Figure 14. Exposed to cold (-5°C). |
| Figure 11. | Figure 15. Oral Zinc at room temperature (20°C). |
| Figure 12. | Figure 16. Oral Zinc exposed to cold (-5°C). |

Plate 3, II.

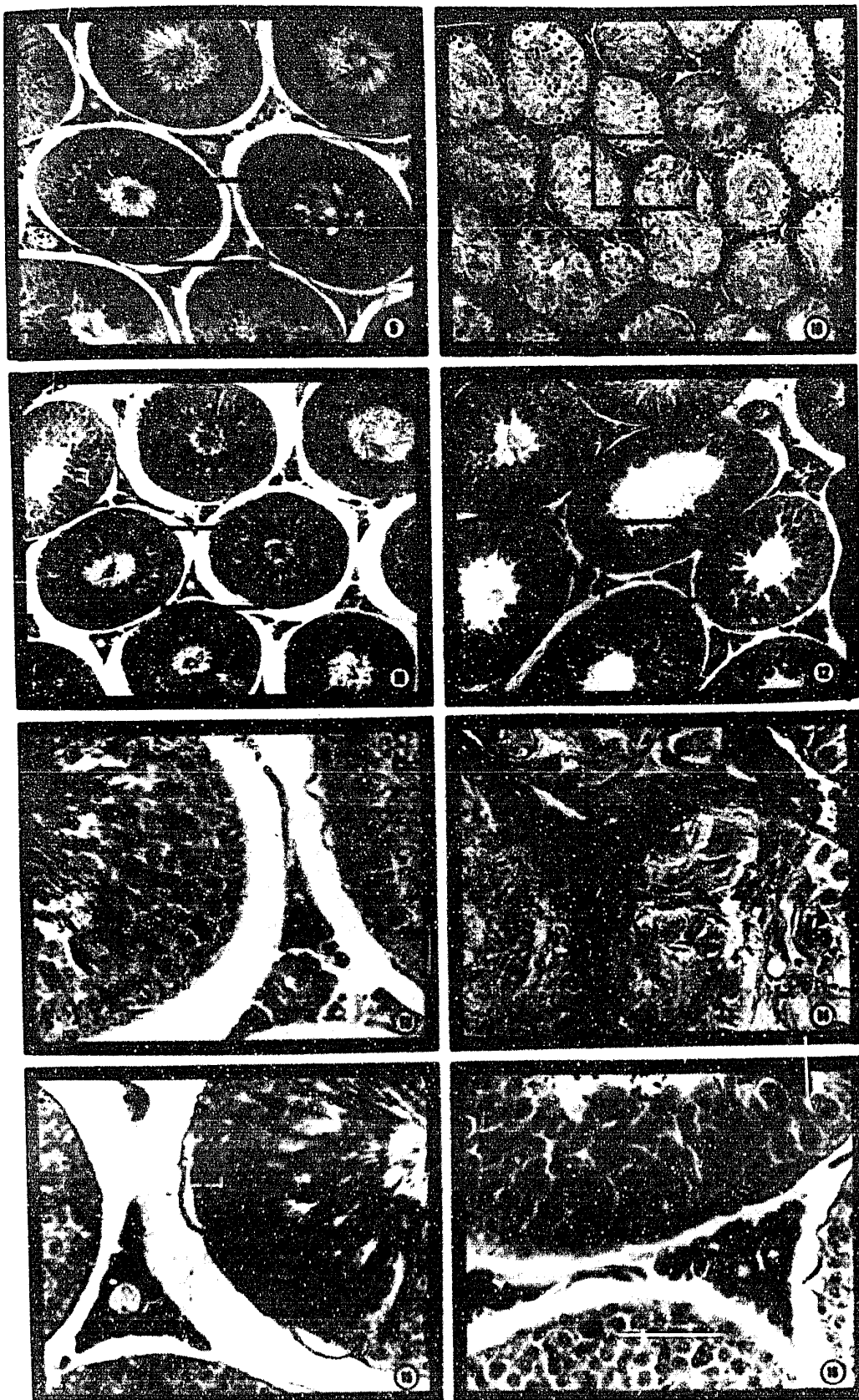


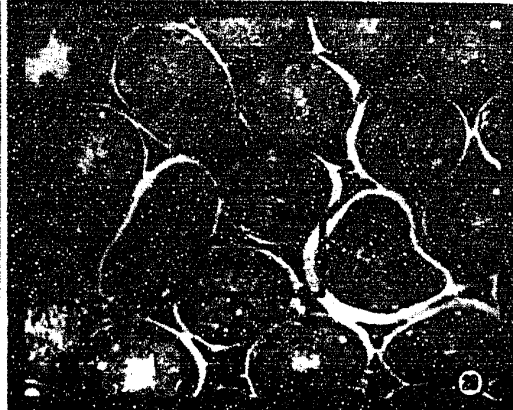
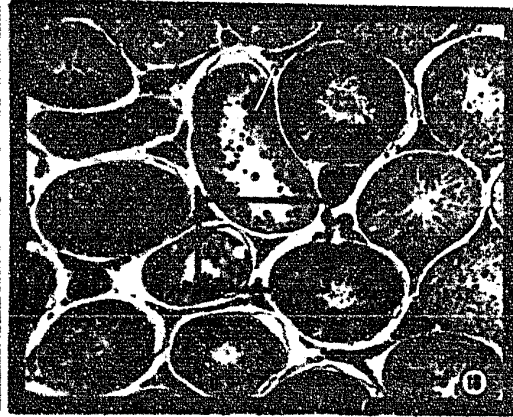
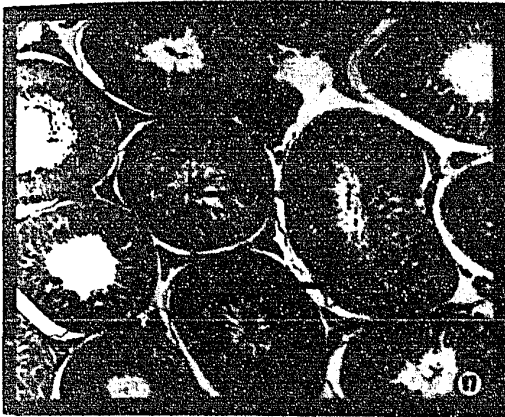
Plate III. Effect of oral Zinc and cold on the testis
of the adult rat; duration of exposure:
50 days (Mallory trichrome).

125 X

500 X

- Figure 17. Figure 21. Control at room temperature (20°C).
Figure 18. Figure 22. Exposed to cold (-5°C).
Figure 19. Figure 23. Oral Zinc at room temperature (20°C).
Figure 20. Figure 24. Oral Zinc exposed to cold (-5°C).

Plate 3, III.



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Chapter Four

THE EFFECT OF ADAPTATION ON THE RESPONSE OF THE
MALE GENITAL APPARATUS TO ACUTE EXPOSURE TO SEVERE COLD (-5°C)

The definition and signs of adaptation to cold have been based on the maintenance of a certain steady level of behavioral, metabolic and morphological characteristics in the animal exposed to cold.

Spontaneous feeding and maintenance of body weight balance (1) allowing temporary oscillation in loss or gain (2) seem to offer the most directly observable information; indications of steadiness come from constancy of colonic temperature and reduction or absence of cold-induced surface injuries (3). Thus, in response to cold stress, a period of resistance first appears, represented by the loss of body weight and variability in survival, followed by gain in body weight among the survivors said to be acclimatized, when the body weight values have returned to the initial value at the time of exposure (4); the process is evidently gradual and can be visualized in the survival rates and body weight curves (5). Adaptation is said to develop slowly, over a period of 5 to 6 weeks at an experimental temperature of 1-3°C, but is lost in a few days after return to neutral temperature environment (6). On a broad conceptual base, animals may be considered adapted if as an individual or as a population, they are capable of living exposed to climactic conditions without artificial aids (7).

Adaptation is the result of a number of functional conditions summed up into "increased energy expenditure" or a "metabolic acclimation" (8), made visible by maintenance of the body core temperature at a

uniform value (9). A more systematic approach recognizes a difference between the situation of the laboratory animal and that of the wild animal in the outdoor climate, and the difference between the animal and the human reactions, where the behavioral pattern becomes highly significant (10, 11, 12).

"Acclimation" or preferably "conditioning to cold" would represent the changes and adjustments in the subject exposed to cold under laboratory conditions (artificially continuous); "Acclimatization" would describe changes (mostly seasonal) in animals exposed to cold in nature and "Adaptation" would include eventual genotypic modifications in species subjected to variations in the climatic environment (13). Intermittent exposure to cold and continuous (laboratory) exposure result in acclimation, but with a difference in degree (14), while wild rats do not display adaptive changes, indicating that the outdoor winter conditions may not be particularly stressful (15).

Assuming that maintenance of body weights, improved survival rates and relative absence of cold injury are signs of some degree of adaptation to continuous (laboratory) exposure to moderate cold (2°C), we wished to know the morphological response of the testicular apparatus of the rat to exposure to severe cold (-5°C) after a period of presumed adaptation.

MATERIALS AND METHODS

Animals: group data were selected from other procedures and special experimental groups set up and arranged according to the norm of Table I, where the season of each test is given; the population and the initial body weights (age) vary among the groups. Exposure was carried out under the usual conditions: ventilated cold rooms thermostatically controlled at 2°C , illumination from 9:00 a.m. to 5:00 p.m. and diet (Purina Lab chow and tap water) ad libitum. Organ weights and microscopic examination provided the desired criteria; frequent weighings and examination for signs of cold injury were carried throughout the procedure.

Since we wished to obtain qualitative data on possible changes in the trends already observed, the statistical evaluation was not carried beyond calculation of the standard error of the mean; values obtained and differences observed were appreciated in relation to the curves of chapter 5.

RESULTS

Body weights (Table I)

The effect of moderate cold is taken from too few subjects to carry actual significance; these values served as a check rather than as an experimental category.

Table I

		Body weights (grams)						
		without adaptation			with adaptation			
		20°C	-5°C	8 days	17 days	+2°C - 16 days -5°C - 7 days	+2°C - 33 days -5°C - 14 days	
Initial		12 days	18 days	8 days	17 days			
Room t°	(1)	292.0	264.3 ± 3.7 ¹ (12) ²	290.0	(1)	263.2 ± 5.7 (10)	266.5 ± 4.0 (13)	200.3 ± 2.0 (15)
Cold	(1)	260.0	(12) 258.2 ± 6.6	(12) 293.7 ± 14.1 (3)	(3)	253.7 ± 5.2 (20)	258.5 ± 5.3 (15)	200.4 ± 1.6 (32)
Post-adaptive								
Room t°	---	---	---	---	---	---	332.2 ± 5.3 (13)	331.4 ± 5.2 (14)
Cold	---	---	---	---	---	---	249.5 ± 5.2 (15)	255.8 ± 5.5 (26)
							-25% (28)	-23% (40)
Terminal								
Room t°	(1)	355.0	(1) 331.0	(2) 350.0	(1)	331.5 ± 6.8 (10)	350.1 ± 6.3 (11)	365.8 ± 2.0 (14)
Cold	(1)	269.0	(1) 250.0	(2) 272.7 ± 17.5 (3)	(3)	216.2 ± 5.7 (20)	260.4 ± 6.2 (11)	252.5 ± 6.3 (25)
	-34% (2) ³		-24% (4)	-22% (4)		-35% (30)	-26% (22)	-31% (39)
(July 17 to July 29) + 20°C	(June 3 to July 1) + 20°C	(July 20 to Aug. 2) -5°C	(Nov. 18 to Dec. 6) -5°C	(June 20 to June 27) 7 days at -5°C	(June 30 to July 6) 14 days at -5°C			

- 1: Standard error of the mean
 2: Group population
 3: Aggregate population

The predictable loss in body weight due to severe cold (-5°C without adaptation) appears as a function of duration, probably independent of age; the terminal body weights of the adapted groups display the usual difference between control and cold-exposed in spite of the presumed adaptation. Comparison of the initial post-adaptive and terminal body weights among the cold-exposed shows that adaptation was successfully accomplished, in that not only the body weight did not fall, but recovery and growth were well marked, provided a sufficient period of time is allowed; the season of the year did not seem to modify visibly the effects observed. Thus, adaptation prevented the absolute fall in body weight consequent to exposure to severe cold, although the difference between controls and exposed is within the predictable range.

Survival rates (Table II)

Survival rates display considerable variations; if the present data are compared with other results, it will be noted that the present 78% survival at 17 days of exposure (-5°C) is the upper value of a wide range of observed survival rates; this may represent a seasonal effect, but one could infer safely that previous adaptation (33 days at 20°C) improved survival, since signs and symptoms of cold injury were actually reduced to a minimum.

Survival rates

(% of initial population)

1: Group population

Organ weights (Table III)

Testes - the loss of weight even in the "adapted", varies with the duration of exposure to severe cold; if reference is made to the prediction curves (Chapter 5), it will be seen that the observed depression corresponds to the exact duration of exposure, irrespective of adaptation. Examination of Table III also shows that exposure to 2°C causes a relatively small depression within the stated period of time.

Seminal vesicles - the same remark applies to the sex indicators; the observed depression is unaffected by the presumed adaptation and falls in the expected range; furthermore, the weight depression due to 2°C exposure is distinctly less marked.

Thymus - the weight depression appears related to intensity of cold per se, and adaptation does not seem to act protectively; the apparent protection of the longer period of adaptation is open to suspicion since the adrenal response proves uniform among all groups.

Adrenals - the adrenal response is surprisingly uniform, irrespective of the intensity or duration of the cold stress; the level of this response is within a narrow margin of the predicted value.

Table III

		Organ weights					
		Without adaptation			With adaptation		
		20°C			-5°C		
		12 days	18 days	8 days	17 days	20°C - 16 days	20°C - 33 days
Testes						-5°C - 7 days	-5°C - 14 days
Room to	3.55 (1) ¹	3.30 (2)	3.56 (1)	3.13 ± 0.10 ³ (10)	3.29 ± 0.14 (11)	3.51 ± 0.11 (14)	
Cold	3.45 (1)	2.92 (2)	2.89 ± 0.11 (3)	2.52 ± 0.10 (20)	2.90 ± 0.35 (12)	2.54 ± 0.04 (24)	
	-3% (2) ²	-11% (4)	-19% (4)	-20% (30)	-12% (23)	-28% (38)	
Seminal vesicles							
Room to	0.67 (1)	0.84 (2)	0.66 (1)	0.62 ± 0.03 (10)	0.67 ± 0.07 (11)	0.58 ± 0.01 (14)	
Cold	0.50 (1)	0.56 (2)	0.39 ± 0.05 (3)	0.24 ± 0.02 (20)	0.40 ± 0.05 (12)	0.25 ± 0.02 (25)	
	-25% (2)	-33% (4)	-42% (4)	-61% (30)	-40% (23)	-57% (39)	
Adrenals							
Room to	36.8 (1)	46.9 (2)	36.6 (1)	39.8 ± 2.2 (10)	43.6 ± 2.2 (11)	44.1 ± 1.3 (14)	
Cold	45.6 (1)	57.6 (2)	46.5 ± 1.7 (3)	47.0 ± 1.2 (20)	52.7 ± 2.9 (12)	53.1 ± 1.7 (25)	
	+19% (2)	+18% (4)	+20% (4)	+15% (30)	+17% (23)	+17% (39)	
Thyroids							
Room to	0.51 (1)	0.44 (2)	0.50 (1)	0.45 ± 0.03 (10)	0.45 ± 0.04 (11)	0.44 ± 0.02 (14)	
Cold	0.29 (1)	0.30 (2)	0.21 ± 0.05 (3)	0.20 ± 0.03 (19)	0.22 ± 0.04 (11)	0.32 ± 0.02 (25)	
	-43% (2)	-32% (4)	-59% (4)	-56% (29)	-51% (22)	-27% (39)	

1: Group population

2: Aggregate population

3: Standard error of the mean

MICROSCOPIC OBSERVATIONS

All illustrations show organs from the "adapted" group:
33 days at 2°C and 14 days at -5°C.

Testes (Figures 1, 2, 13, 14).

A certain degree of degeneration of the germinal tissue and of the interstitium is visible; however, the tubular diameter is greater than previously seen and some tubules contain intact spermatogenic sequences. This feature is particularly evident in comparison with the considerable quiescence of the control; qualitatively the present testis looks considerably better than expected.

Prostate (Figures 3, 4, 15, 16).

These images display the classical regression of prostatic epithelium, characteristic of severe depression of androgenicity almost to the level of the castrate.

Seminal vesicles (Figures 5, 6, 17, 18).

This picture of degeneration is less dramatic than that of the prostate; this would be consistent with the belief of some endocrinologists that the vesicles possess a lower threshold of sensitivity to androgens than the prostate and thus would respond to lower titers of male hormones.

Adrenals (Figures 7, 8, 19, 20).

A low magnification gives a complete view of the cortex; the hypertrophy here seen is identical with that observed previously and the adaptation period does not seem to make the adrenal response any different from the response to acute exposure.

Thymus (Figures 9, 10, 21, 22).

The thymic lobules of the control show nicely the contrast between medulla and cortex: the cortex with its dustlike thymocytes arranged along a vague reticular background and the faintly staining medulla with its scarce lymphocytes and prominent reticular stroma. As often stated, no feature of its minute structure suggests a glandular function. The cold-exposed thymus retains its lobular architecture, but presents a uniform stroma where one of the structural divisions has disappeared, probably the medulla; no evidence is seen of the Hassall's corpuscles said to represent adult shrivelling of the medulla in the human gland. We do not know if this picture is typical of the stress response and the significance of the changes: loss of constituents or depletion of "product"? Once more, the elusiveness of the thymus remains inviolate.

Pituitary (Figures 11, 12, 23, 24).

The pituitary cytology presents a hazardous field for the unwary; in the cold-exposed subject, the optimistic observer might see an increase in the number of basophils with shapes strangely reminiscent of the castration "signet ring" cells, suggesting a disturbance in gonadotropin production and/or release.

DISCUSSION

Superficial as these observations may be, more problems are posed than are resolved. The body weights, growth pattern and external appearance gave every indication of some degree of acquired resistance; however, functional criteria seem to indicate that the resistance does not extend to the reproductive apparatus. If the adrenal response is assumed to be a sign of non-adaptation, then the hypertrophy could be interpreted as a qualitative deficit of cortical hormones which normally would favor acclimation, but only as a second line of defence (16); this would confirm the cautious statement that physiological adjustments reflected in the morphology represent continuous variables of complex reactive origins (17) and of different degrees of plasticity. Perhaps, the recognized pattern (i.e. the sum total of the adaptive responses by which the animal can survive in the cold and maintain body temperature (18) where the unacclimatized would go into hypothermia (19)) does not tell the complete story of the endocrine responses, especially in areas less directly involved in the balance of energetic exchanges; such would be the reproductive apparatus where the functional level receives no protection consequent to adaptation.

Would these observations point to a dissociation between somatic and endocrine levels of activity, where the purely endocrine control would be primary and the metabolic factors secondary and subordinate? Perhaps the concept of physiological castration as ransom of the male survival could shed light on the stress response of the testicular apparatus (20).

Plate I. Effect of severe cold on the genital apparatus of the "adapted" adult male rat (125 X - Mallory trichrome).

Figure 1. Testis - control at room temperature (20°C).

Figure 2. Testis - exposed to cold (-5°C).

Figure 3. Ventral prostate - control (20°C).

Figure 4. Ventral prostate - exposed to cold (-5°C).

Figure 5. Seminal vesicle - control (20°C).

Figure 6. Seminal vesicle - exposed to cold (-5°C).

Plate 4, I.

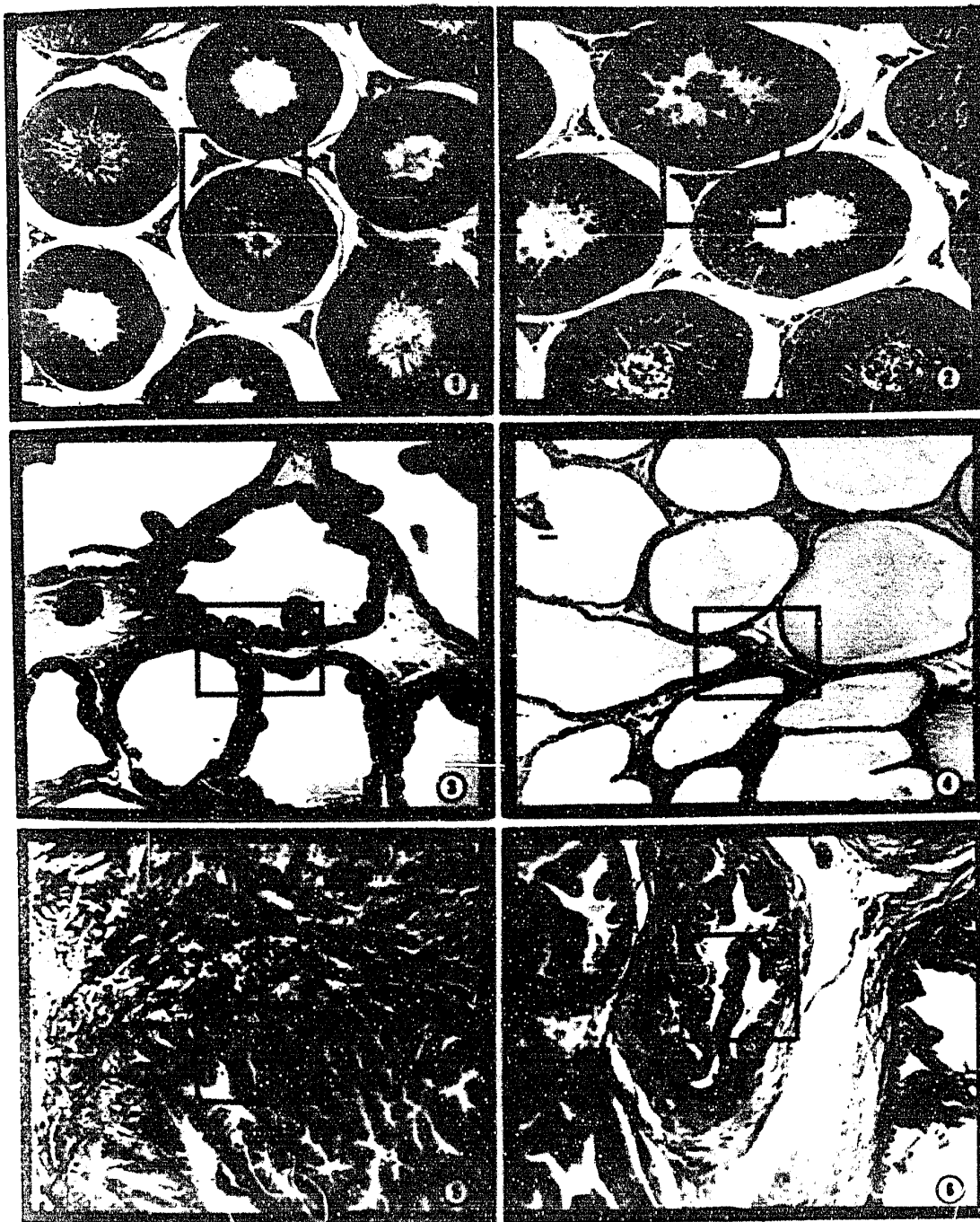


Plate II. Effect of severe cold on some endocrine organs of the
"adapted" adult male rat (Mallory trichrome).

Figure 7. Adrenal - control at room temperature (20°C). 37X

Figure 8. Adrenal - exposed to cold (-5°C). 37X

Figure 9. Thymus - control (20°C). 37X

Figure 10. Thymus - exposed to cold (-5°C). 37X

Figure 11. Pituitary - control (20°C). 500X

Figure 12. Pituitary - exposed to cold (-5°C). 500X

Plate 4, II.

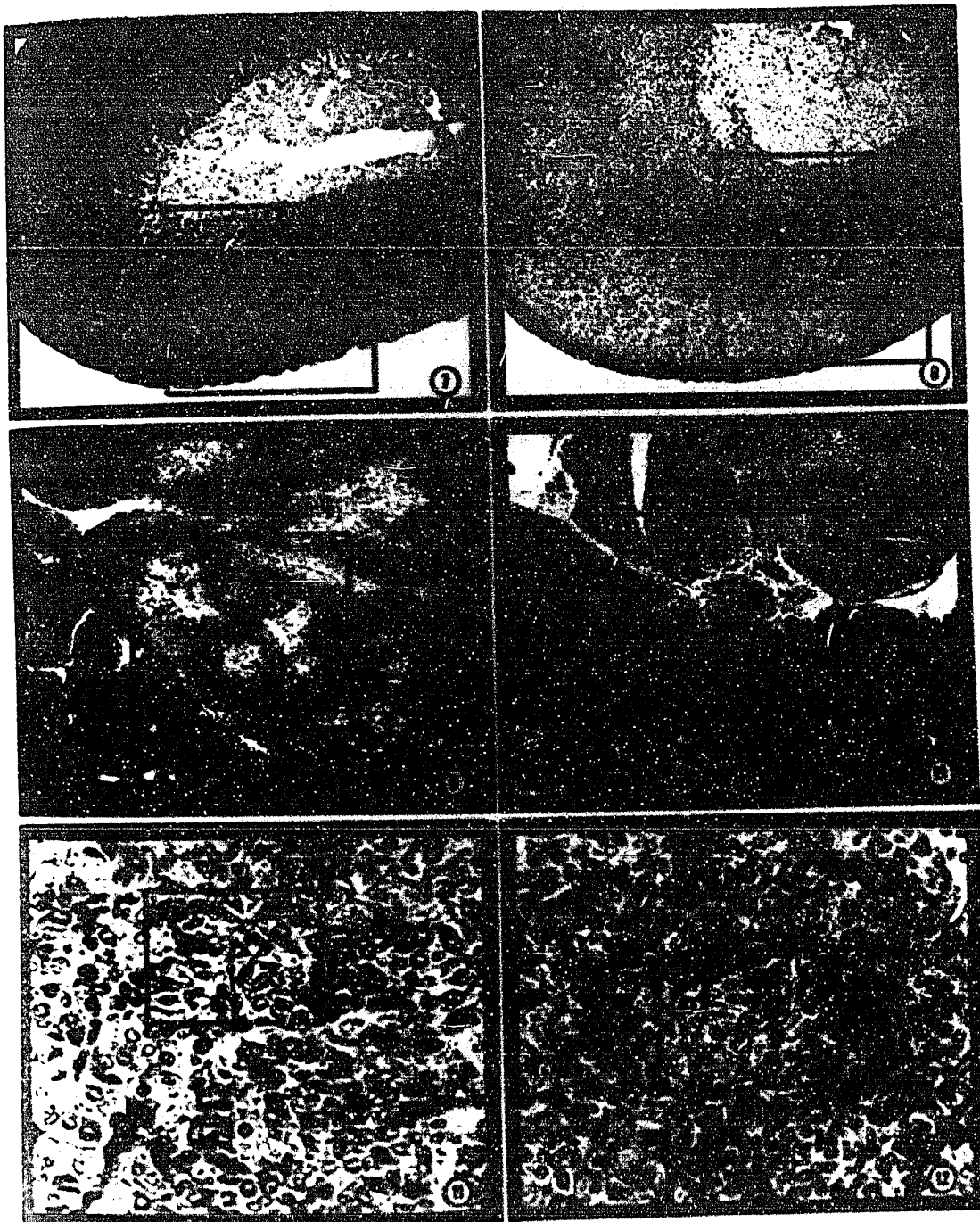


Plate III. Effect of severe cold on the genital apparatus of the "adapted" adult male rat (500 X - Mallory trichrome).

Figure 13. Testis - control at room temperature (20°C).

Figure 14. Testis - exposed to cold (-5°C).

Figure 15. Ventral prostate - control (20°C).

Figure 16. Ventral prostate - exposed to cold (-5°C).

Figure 17. Seminal vesicle - control (20°C).

Figure 18. Seminal vesicle - exposed to cold (-5°C).

Plate 4, III.

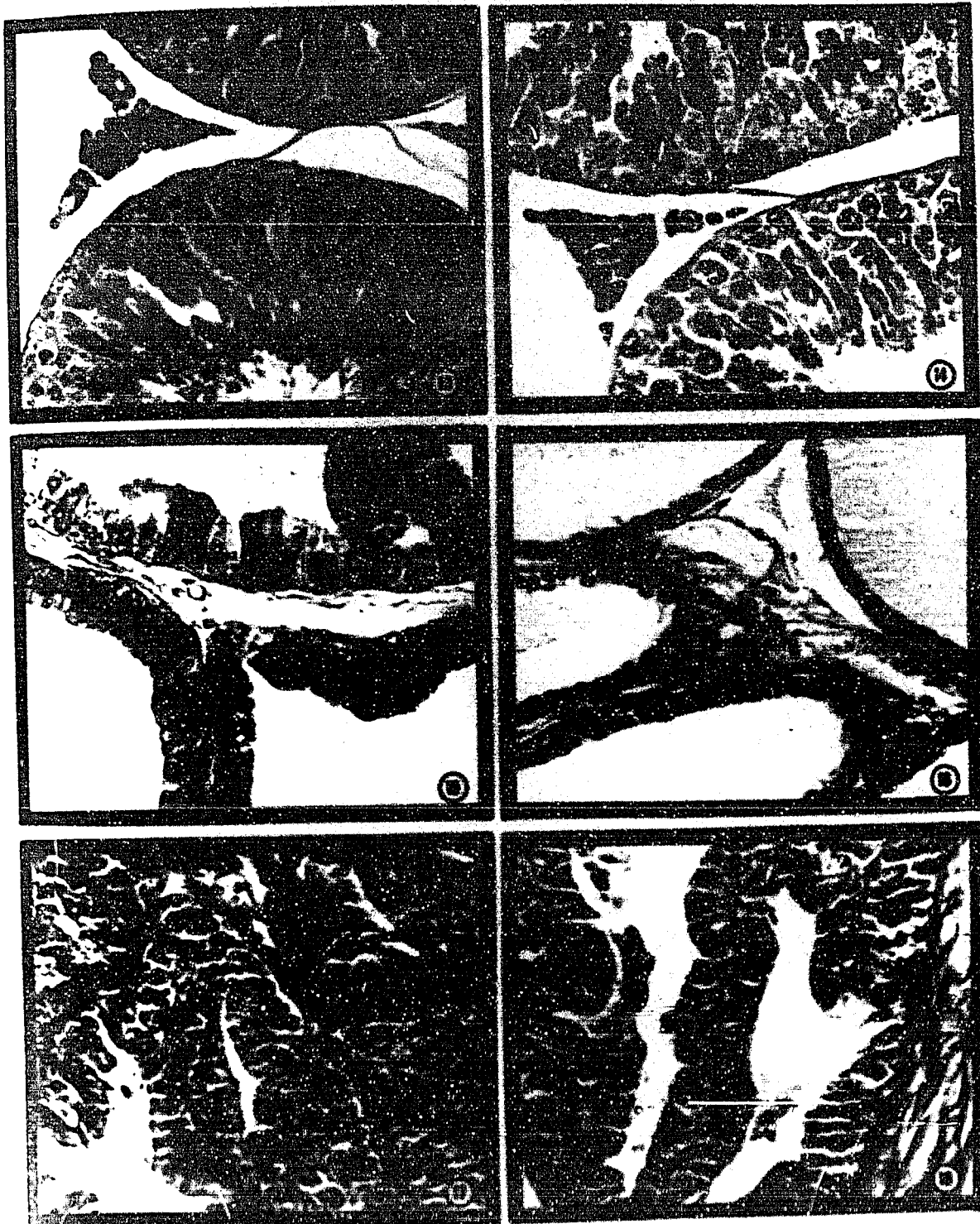


Plate IV. Effect of severe cold on some endocrine organs
of the "adapted" adult male rat (Mallory trichrome).

Figure 19. Adrenal - control at room temperature (20°C). 75X

Figure 20. Adrenal - exposed to cold (-5°C). 75X

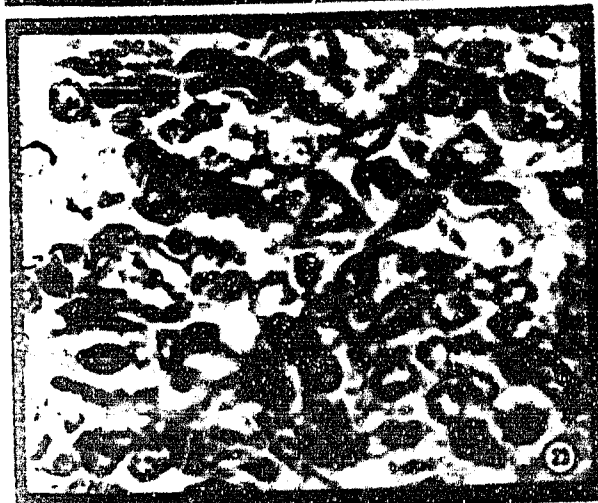
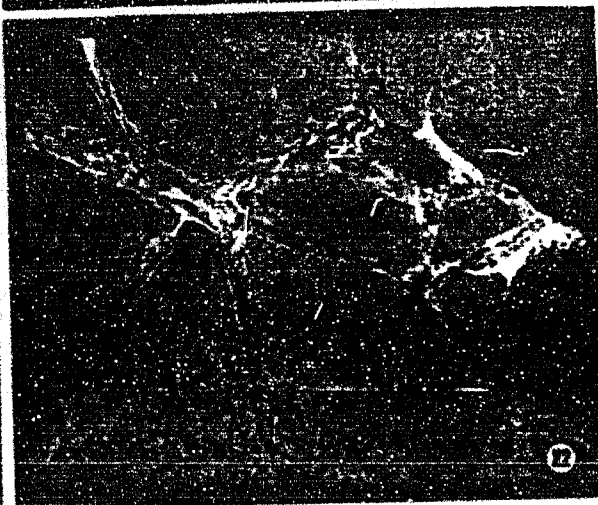
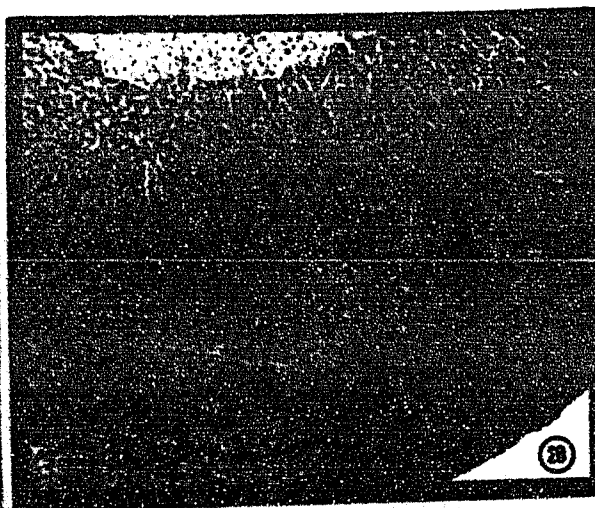
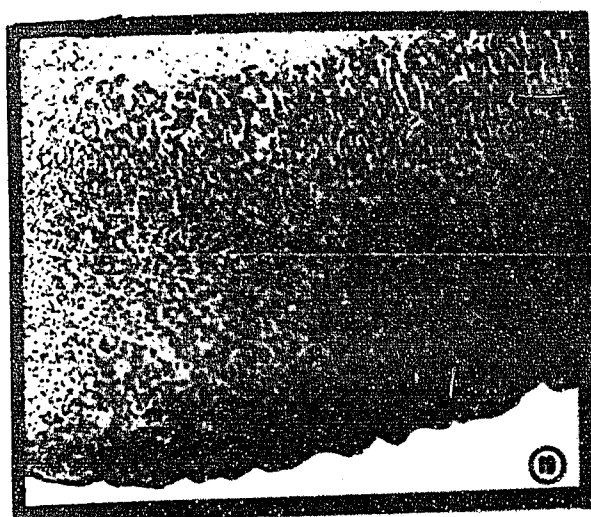
Figure 21. Thymus - control (20°C). 75X

Figure 22. Thymus - exposed to cold (20°C). 75X

Figure 23. Pituitary - control (20°C). 1250X

Figure 24. Pituitary - exposed to cold (-5°C). 1250X

Plate 4, IV.



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Chapter Five

TIME SCHEDULE OF TESTICULAR DAMAGE INDUCED BY
SEVERE COLD (-5°C)

The degeneration of the testicular function induced by chronic exposure to moderate cold (2°C) can be duplicated in effect and compressed in time if the temperature is lowered to -5°C . Preceding chapters contain data obtained at various time points; if a dynamic view is to be secured, a schedule should show the onset, development and time-course of some of the observed characteristics. A procedure was designed combining two parameters: duration of exposure and difference in the age of the animals. Since the large number of subjects precluded the use of litter mates, body weight was taken as a reasonable criterion of age; thus, animals of the same average body weight and reared under identical conditions, were assumed to fall within a narrow margin of age. By sacrifice at short time intervals, the observed changes could be referred to a time sequence and trends verified.

MATERIALS AND METHODS

A random group of male rats (Wistar Albino) was arranged according to the norm of Table I; no treatment was administered, only the usual 5 to 6-day quarantine preceded entry into the cold. The subjects were caged individually in air conditioned quarters maintained at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until exposition to cold ($-5^{\circ}\text{C} \pm 1^{\circ}\text{C}$); the ad libitum diet consisted of Purina lab chow and tap water.

Table I

	Jan. 6 - Jan. 22	Jan. 31 - Feb. 23	May 30 - July 4
20°C	22*	24	15
-5°C	33	35	21

*: Group population

The dates indicate the season during which the cold exposure was accomplished; evidently, the animals represent three different groups, but Figure 1 shows excellent continuity of development between the groups and permits the assumption that the subjects form a fairly homogeneous population. The initial body weights (assumed to represent the age) are different, so that a comparison of response in terms of age could be obtained.

Every 2 days from the beginning of exposure, 2 controls and 3 cold-exposed were sacrificed and autopsied; since the data from the casualties were almost always compatible with the autopsy data, the figures were combined to represent that time-point. The results were arranged in 2 different ways: the 10-day intervals present the pooled data in table form; a curve equation (method of means) was obtained from the individual 2-day values.

Autopsy data. The usual organ weights were obtained, total cholesterol determinations were made on the testes and microscopic sections of testes from each 2-day group were prepared.

Statistical treatment. All values were screened by means of the Dixon test for extremes; means are always quoted with the standard error. Only the Student's T test was applied to control versus cold-exposed for each 10-day point. Figures 2 and 3 were obtained as follows: the % difference between control and cold-exposed for each 2-day point, was plotted against duration of exposure as a plus or a minus deviation from a baseline assumed to represent the control value and by inspection the solid line was traced. Weighted means were obtained for the time points and the curve equations were calculated; the mathematical functions were traced on cartesian coordinates (broken line) on figures 2 and 3. Figure 4 shows the same equations on a semi-logarithmic scale resulting in the usual straight lines. For the sake of clarity, the clouds of individual points are omitted and only the curves are shown in figures 2 and 3. The curves are made to start at the origin although data for an exposure period of less than 2 days are not sufficiently reliable; figure 4 leaves blank this uncertain area. The dotted circles represent loci of data to be discussed in the next chapter.

RESULTS

Body weights (Table II, Figures 1, 3 and 4).

The depression of the body weights is fairly similar in all age groups; it appears independent of age and related only to the duration of exposure. A minimal dispersion of individual points shows continuity in the time development of the overall body weight depression.

Table II

Body weights				
Time of experiment				
	Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4	
Initial				
20°C	218.4 ± 2.8 ¹ (22) ²	262.4 ± 2.5 (24)	373.6 ± 4.4 (15)	
-5°C	217.3 ± 2.4 (33)	260.1 ± 2.3 (35)	338.2 ± 8.2 (21)	
			<u>-10%</u> (36) ³	
Terminal	<u>0 to 10 days</u>			
20°C	243.6 ± 7.3 (10)	293.4 ± 6.7 (12)	391.3 ± 10.3 (3)	
-5°C	195.3 ± 4.4 (18)	238.3 ± 5.6 (19)	284.5 ± 16.1 (4)	
	<u>-20%</u> (28)	<u>-19%</u> (31)	<u>-27%</u> (7)	
	<u>11 to 20 days</u>			
20°C	290.6 ± 5.0 (12)	331.5 ± 5.4 (12)	419.2 ± 8.1 (6)	
-5°C	199.5 ± 4.8 (15)	226.3 ± 5.7 (19)	295.6 ± 8.2 (7)	
	<u>-31%</u> (27)	<u>-32%</u> (27)	<u>-29%</u> (13)	
	<u>21 to 30 days</u>			
20°C	-----	-----	408.2 ± 6.1 (6)	
-5°C	-----	-----	245.4 ± 3.6 (8)	
			<u>-40%</u> (14)	

1: Standard error of the mean

: p < 0.001

2: Group population

3: Aggregate population

Growth curves (Figure 1). Samples of growth curves after smoothing out of daily variations, display uniform rates of growth for the various groups; this semi-logarithmic pattern is familiar to all experimenters cognisant of the age-weight relationship in the rat. The survival rates show the usual dependency upon the duration of exposure and do not appreciably differ from the values quoted previously, i.e. a 50% mortality is attained at approximately 18 days.

Organ weights and other data.

Testes (Table III, Figures 2 and 4). The changes in testes weight follow a gradual pattern, where loss becomes proportional to the duration of exposure and parallels the loss in body weight.

Table III

Testes (weight in grams)			
Time of experiment			
	Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4
0 to 10 days			
20°C	2.78 ± 0.08 ¹ (10) ²	3.09 ± 0.03 (12)	3.29 ± 0.10 (3)
-5°C	2.58 ± 0.06 (18)	2.73 ± 0.07 (19)	2.97 ± 0.20 (4)
	<u>-10%</u> (28) ³	<u>-12%</u> (31)	<u>-10%</u> (7)
11 to 20 days			
20°C	3.09 ± 0.07 (12)	3.21 ± 0.09 (12)	3.27 ± 0.03 (5)
-5°C	2.47 ± 0.07 (15)	2.40 ± 0.10 (15)	2.67 ± 0.02 (8)
	<u>-20%</u> (27)	<u>-25%</u> (27)	<u>-18%</u> (13)
21 to 30 days			
20°C	—	—	3.19 ± 0.01 (4)
-5°C	—	—	2.66 ± 0.11 (10)
			<u>-17%</u> (14)

- 1: Standard error of the mean — —: p < 0.05
 2: Group population — —: p < 0.01
 3: Aggregate population — —: p < 0.001

The total testis cholesterol (Table IV, Figures 2' and 4), shows considerable dispersion; nevertheless, relative increased production appears fairly evident in relation to the duration of exposure; worthy

of note is the delay in the theoretical induction of the increased production. The slight variation due to age reported in the previous chapter can also be observed here.

Table IV

Total testis cholesterol				
(mg % gms wet tissue)				
Time of experiment				
	Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4	
0 to 10 days				
20°C	150.3 ± 7.3 ¹ (10) ²	125.3 ± 6.3 (12)	126.5 ± 5.3 (3)	
-5°C	163.1 ± 3.9 (17)	154.1 ± 7.7 (19)	137.6 ± 11.2 (4)	
	+8% (27) ³	+18% (31)	+8% (7)	
11 to 20 days				
20°C	144.6 ± 6.9 (12)	144.8 ± 6.4 (12)	147.7 ± 15.4 (6)	
-5°C	155.6 ± 5.3 (15)	179.9 ± 7.7 (15)	169.2 ± 15.6 (8)	
	+7% (27)	+19% (27)	+12% (14)	
21 to 30 days				
20°C	-----	-----	117.4 ± 0.8 (2)	
-5°C	-----	-----	130.2 ± 13.4 (4)	
			+10% (6)	

1: Standard error of the mean

—: p < 0.01

2: Group population

3: Aggregate population

Sexual accessories (Tables V and VI - Figures 2, and 4).

The depression in the weight of these organs is proportional to the duration of exposure, irrespective of age; worthy of note is the fact that this depression is far below the level observed in the depression of the body weights and of the testes weight. The onset is precipitous and identical in the prostate and the vesicles; the trend eventually levels off.

Table V

Prostate (weight in grams)			
Time of experiment			
	Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4
0 to 10 days			
20°C	0.24 ± 0.03 ¹ (10) ²	0.40 ± 0.02 (12)	0.55 ± 0.05 (3)
-5°C	0.14 ± 0.01 (17) <u>-42%</u> (27) ³	0.28 ± 0.02 (19) <u>-30%</u> (31)	0.41 ± 0.03 (4) <u>-25%</u> (7)
11 to 20 days			
20°C	0.41 ± 0.02 (12)	0.43 ± 0.02 (12)	0.50 ± 0.04 (6)
-5°C	0.16 ± 0.01 (15) <u>-61%</u> (27)	0.23 ± 0.02 (15) <u>-47%</u> (27)	0.32 ± 0.02 (8) <u>-36%</u> (14)
21 to 30 days			
20°C	---	---	0.57 ± 0.08 (6)
-5°C	---	---	0.20 ± 0.03 (10) <u>-65%</u> (16)

1: Standard error of the mean

—: p < 0.05

2: Group population

—: p < 0.01

3: Aggregate population

—: p < 0.001

Table VI

Seminal vesicles (weight in grams)				
Time of experiment				
	Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4	
0 to 10 days				
20°C	0.29 ± 0.05 ¹ (9) ²	0.45 ± 0.02 (12)	0.64 ± 0.08 (3)	
-5°C	0.15 ± 0.01 (18)	0.30 ± 0.02 (19)	0.45 ± 0.03 (4)	
	<u>-48%</u> (27) ³	<u>-35%</u> (31)	<u>-30%</u> (7)	
11 to 20 days				
20°C	0.46 ± 0.02 (12)	0.46 ± 0.02 (12)	0.63 ± 0.03 (6)	
-5°C	0.17 ± 0.02 (15)	0.24 ± 0.02 (15)	0.39 ± 0.03 (8)	
	<u>-63%</u> (27)	<u>-48%</u> (27)	<u>-38%</u> (14)	
21 to 30 days				
20°C	---	---	0.60 ± 0.04 (6)	
-5°C	---	---	0.28 ± 0.01 (10)	
			<u>-53%</u> (16)	

1: Standard error of the mean

---: p < 0.05

2: Group population

---: p < 0.01

3: Aggregate population

---: p < 0.001

Stress indicators.

Adrenals (Table VII, Figures 3 and 4). In spite of considerable dispersion, a continuity can be observed in the level of the adrenal response to the duration of the cold exposure.

Table VII

Adrenals (weight in milligrams)			
Time of experiment			
	Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4
0 to 10 days			
20°C	36.7 ± 1.6 ¹ (9) ²	37.1 ± 1.4 (12)	43.1 ± 1.5 (3)
-5°C	46.6 ± 2.2 (18)	48.1 ± 1.3 (19)	52.1 ± 3.9 (4)
	+21% (27) ³	+23% (31)	+17% (7)
11 to 20 days			
20°C	38.9 ± 1.1 (12)	41.7 ± 1.2 (12)	43.1 ± 0.9 (6)
-5°C	52.2 ± 2.0 (15)	55.4 ± 1.6 (15)	55.8 ± 1.9 (8)
	+25% (27)	+25% (27)	+23% (14)
21 to 30 days			
20°C	---	---	46.1 ± 2.6 (6)
-5°C	---	---	65.1 ± 2.7 (10)
			+29% (16)

1: Standard error of the mean

---: p < 0.01

2: Group population

---: p < 0.001

3: Aggregate population

Thymus (Table VIII, Figures 3 and 4). The thymus shows dispersion in the later stages of the curve, but the early response is uniformly precipitous irrespective of age; the trend levels off eventually.

Table VIII

Thymus (weight in grams)				
Time of experiment				
		Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4
0 to 10 days				
20°C	0.40 ± 0.04 ¹ (10) ²	0.40 ± 0.03 (12)	0.53 ± 0.02 (3)	
-5°C	0.20 ± 0.02 (18)	0.19 ± 0.02 (19)	0.16 ± 0.02 (4)	
	<u>-50%</u> (28) ³	<u>-52%</u> (31)	<u>-70%</u> (7)	
11 to 20 days				
20°C	0.38 ± 0.02 (11)	0.41 ± 0.02 (12)	0.48 ± 0.06 (6)	
-5°C	0.14 ± 0.01 (15)	0.11 ± 0.01 (15)	0.10 ± 0.02 (8)	
	<u>-63%</u> (26)	<u>-73%</u> (27)	<u>-79%</u> (14)	
21 to 30 days				
20°C	---	---	0.45 ± 0.04 (6)	
-5°C	---	---	0.10 ± 0.02 (10)	
			<u>-78%</u> (16)	

1: Standard error of the mean

---: p < 0.001

2: Group population

3: Aggregate population

Miscellaneous data.

Pituitary (Table IX, Figures 3 and 4). These data, few in number, reveal some continuity in the weight depression; the superficial observer could perhaps see the definite relationship displayed optimistically in Figure 4.

Table IX

Pituitary				
(weight in milligrams)				
Time of experiment				
	Jan. 6 to Jan. 22	Jan. 31 to Feb. 23	May 30 to July 4	
0 to 10 days				
20°C	-----	9.5 ± 0.3 ¹ (12) ²	10.4 ± 0.5 (3)	
-5°C	-----	8.9 ± 0.2 (18)	8.7 ± 0.5 (4)	
		-6% (30) ³	<u>-16%</u> (7)	
11 to 20 days				
20°C	9.8 ± 0.1 (3)	10.4 ± 0.3 (12)	11.7 ± 0.3 (5)	
-5°C	7.9 ± 0.3 (2)	8.0 ± 0.3 (15)	9.7 ± 0.3 (8)	
	<u>-19%</u> (5)	<u>-26%</u> (27)	<u>-17%</u> (13)	
21 to 30 days				
20°C	-----	-----	11.2 ± 0.8 (6)	
-5°C	-----	-----	9.2 ± 0.4 (10)	
			<u>-18%</u> (16)	

1: Standard error of the mean --: p < 0.05

2: Group population

3: Aggregate population ==: p < 0.001

Liver and lungs (Table I). The lungs remain essentially unchanged; the liver seems to suffer a temporary depression, followed by a tendency to return to normal.

Table X

	Liver (weight in grams)	Lungs (weight in grams)
Time of experiment: May 30 to July 4		
0 to 10 days		
20°C	12.6 ± 0.01 ¹ (3) ²	1.52 ± 0.10 (3)
-5°C	11.4 ± 0.6 (4) -9% (7) ³	1.37 ± 0.02 (3) -10% (6)
11 to 20 days		
20°C	15.4 ± 0.6 (6)	1.50 ± 0.09 (6)
-5°C	11.7 ± 0.7 (8) -21% (14)	1.49 ± 0.03 (8) -1% (14)
21 to 30 days		
20°C	15.8 ± 0.6 (6)	1.45 ± 0.05 (6)
-5°C	13.4 ± 0.7 (10) -15% (16)	1.36 ± 0.04 (7) -6% (13)
1: Standard error of the mean —: p < 0.05 2: Group population —: p < 0.01 3: Aggregate population		

Water contents (Table XI). The constancy of organ composition insofar as it is indicated by water contents, resists duration of exposure, irrespective of age.

Table XI

% Water contents			
	Testes	Prostate	Seminal vesicles
0 to 10 days			
20°C	-----	85.7 ± 2.0^1 (3) ²	77.3 ± 0.8 (3)
-5°C	-----	85.4 ± 1.2 (4)	80.7 ± 0.3 (3) + 4% (6) ³
11 to 20 days			
20°C	-----	82.2 (6)	77.6 (6)
-5°C	-----	83.7 (8) + 2% (14)	79.7 (6) + 3% (12)
21 to 30 days			
20°C	87.0 ± 0.0 (3)	84.5 (5)	78.6 (6)
-5°C	86.7 ± 0.2 (6)	85.3 (9) + 1% (14)	82.3 (10) + 4% (16)

1: Standard error of the mean

2: Group population

3: Aggregate population

Table XII

% Water contents				
	Adrenals	Thymus	Liver	Lungs
0 to 10 days				
20°C	74.3 ± 0.6 ¹ (3) ²	80.0 ± 0.6 (3)	70.2 ± 0.5 (2)	78.3 ± 0.1 (3)
-5°C	75.6 ± 2.0 (4) +3% (7) ³	78.3 ± 0.3 (3) -2% (6)	72.3 ± 0.3 (3) +3% (5)	78.4 ± 1.7 (4)
11 to 20 days				
20°C	73.0 (6)	76.7 (6)	71.0 (6)	76.5 (6)
-5°C	69.1 (8) -5% (14)	76.9 (7)	70.8 (7)	77.4 (8)
21 to 30 days				
20°C	74.5 (6)	79.9 (5)	72.8 (6)	78.4 (5)
-5°C	71.3 (10) -4% (16)	77.4 (9) -3% (14)	75.9 (10) +4% (16)	79.4 (9) +1% (14)

1: Standard error of the mean

2: Group population

3: Aggregate population

Microscopic observations. Examination of slides of testes from each of the 2-day stages with the avowed bias of finding specific progressive changes, did not yield more than was reported in the previous chapters; alterations to the germinal sequence became observable at approximately 8 days, but admit considerable variation in the time of onset and extent of damage. Evaluation of the modifications to the interstitial spaces proved once more, unreliable, debatable and diagnostically inconclusive.

The clumping of the spermatids, evidently a degenerative feature, could perhaps be analogized to the Hassall's corpuscles of the aging thymus and to the "epithelial pearls" of skins carcinomas.

DISCUSSION

1. The How and the Why of the curves (Figures 2, 3 and 4).

Various methods were tried of expressing in a consistent form the results of the present investigation. Plots were made of absolute values of controls and cold-exposed against such parameters as season of the year and body weights (initial or terminal); the use of these independent variables proved unsatisfactory. From inspection of the data, the duration of exposure, irrespective of age or season, seemed to possess reasonable continuity; the % difference between control and experimental was therefore plotted against duration of exposure and yielded a more logical distribution of points.

The calculated curve (broken line) coincided rather well with the curve drawn from a strictly visual inspection (solid line), where the french curve is made to follow the largest concentration of points (Figures 2 and 3). The tracings resulting from this treatment permitted evaluation of the data from the present and from the preceding procedures. We did not feel justified in pressing further such congenitally imprecise data; indeed, the aim was not the measure of the variability of the response (deviation of values from calculated curve), but to find some form of expression of the basic trend in the response. Although provisional and qualitative, these curves served as a basis of comparison whereby the overall response could be broken down into potential components.

2. Effect of severe cold on the soma. The emaciation observed engages at various degrees the different tissues of the body; this overall

deterioration represents the plastic components only and approaches a limit made up of the indestructible structures of the body. Indeed, under severe cold, only a few sturdy individuals survive and adaptation may well be considered near to impossible. The body weight depression appears as a function of duration of the cold stress, probably independent of age and season; the statistical significance appears at 16 days.

3. Effect of severe cold on the testis. Observations on the weight depression of the testes (significant at 12 days), tend to show proportionality with body weight losses; thus, testis weight could perhaps be considered as a somatic property as would be the weight characteristics of the pituitary (change significant at 18 days). Other data lead us to suspect that the young testis may be more sensitive to cold than the older organ; the difference might be more apparent because of the more rapid rate of growth in the younger organism. Reliable data on the functional changes of the endocrine testis must therefore come from other criteria, such as the changes in the sexual accessories.

The weight changes in the accessories are definitely out of proportion with the somatic modifications and thus appear to relate in great part to the level of endocrine activity of the testis. The more sensitive prostate displays significant depression after 6 days of exposure and the seminal vesicles after 10 days. Therefore, the changes in the accessories would be due in part to somatic alterations and in part to true endocrine hypofunction.

Assuming that total testis cholesterol is an index of androgenic activity, the observer could provisionally see an inverse proportionality between testis function and cholesterol contents, confirming the significant depression of endocrine activity of the testis.

4. Effect of severe cold on the Stress indicators. The adrenals react by an increase in weight confirmed by hypertrophy and hyperplasia, visible especially in the fasciculata; this response is proportional to the duration of exposure and within our conditions, independent of age. The relatively long intervals do not show the effect of the initial hours following entry into the cold, nor the possible periodicity of function. Previous observations have shown that after 1 hour at -5°C , the adrenal weight drops (5% but significant); later, the weight increases gradually with sporadic significance (significant at 9 and 36 hours; not significant at 12, 24 and 72 hours). This suggests that the story of the stressed adrenal is far more complex than the present results would show and that some period of oscillation may precede the gross and massive response which becomes significant at 4 days; furthermore, the adrenal androgens may well become a significant factor in the economy of the organism under stress.

Thymolysis, significant at 7 days, appears dependent upon the duration of stress rather than on age; however, the younger animal shows earlier and more marked thymolysis. This reduction in thymus weight is out of proportion with the somatic changes, develops precipitously in the early period of the stress response, arguing in favor

of the indicator function of the thymus relative to adrenal function.

CONCLUSIONS

In view of the present results, certain conclusions can be provisionally accepted.

1. The somatic changes due to cold (emaciation) are directly proportional to the duration of stress, rather than to age; these somatic changes are limited by the very structure of the body.
2. The weight depression of a primary endocrine organ, e.g. testis, pituitary, is not per se a reliable criterion of functional depression, since the weight loss is in the same order of values as the somatic depression.
3. The stress response of the adrenal is unrelated to age, but appears proportional to the duration of stress, at a given value of stress intensity and within a reasonable age range.
4. The weight depression of secondary endocrine organs, sexual accessories and thymus, is a valid indicator of functional changes in the primary organ; this indicator value lies in the magnitude of the weight change beyond somatic effects.
5. The overall changes observed in the endocrine indicators (sexual accessories and thymus) consist of 2 components: somatic, related to metabolic alterations engaging the whole organism and specific, consequent to changes in the functional level of the primary organ.
6. The morphological changes are valid indicators of functional

changes due to stress only insofar as experimental is opposed to control under identical conditions of age, season and treatment.

7. Age per se does not seem to alter qualitatively the stress response; within the present range no effect could be detected.

8. Although provisional the present observations give a fairly consistent picture of anatomical and functional reaction of the testicular function to severe cold; further experimentation will elucidate the components of this complex response.

Figure 5,1.

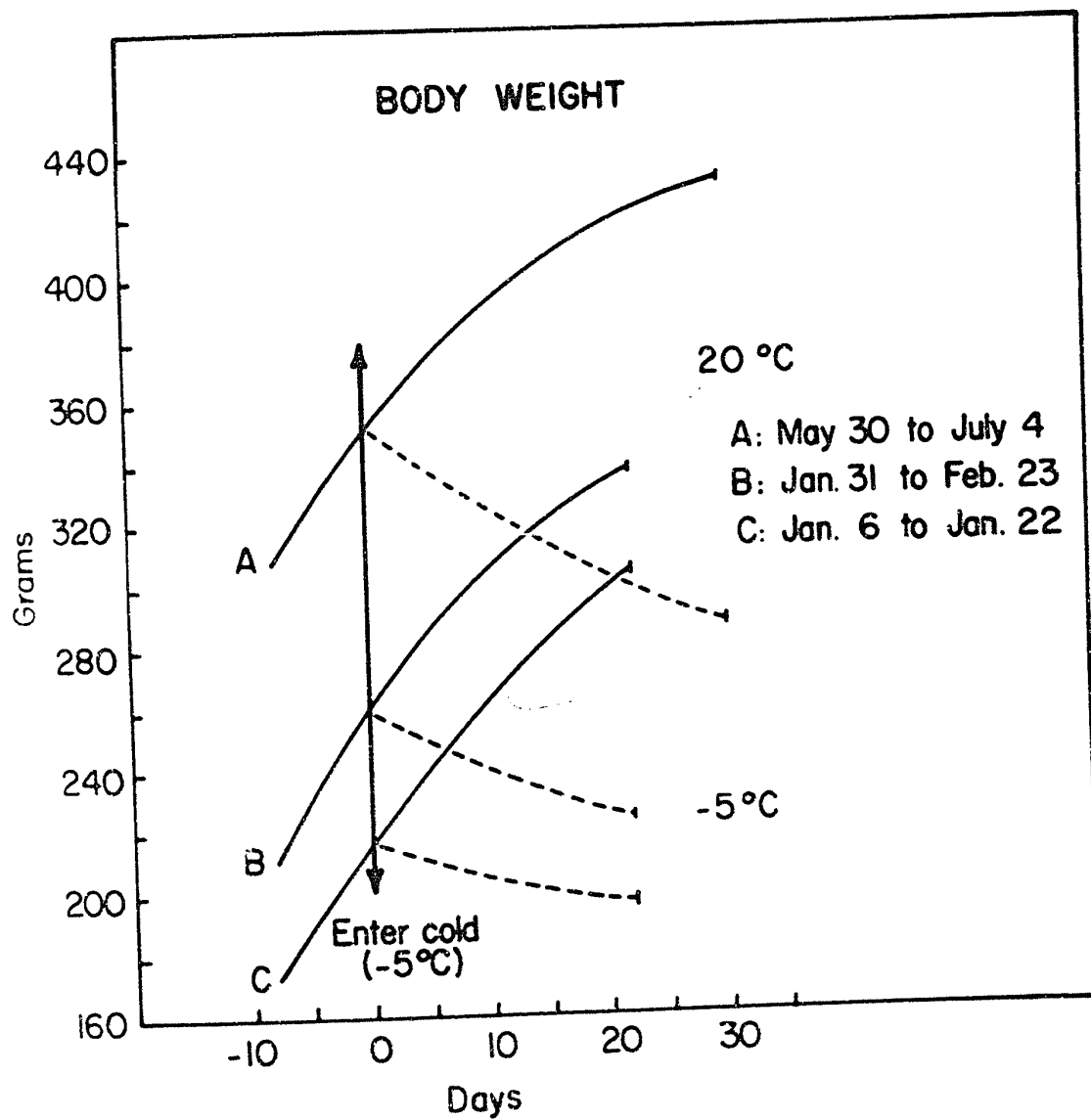


Figure 5, 2.

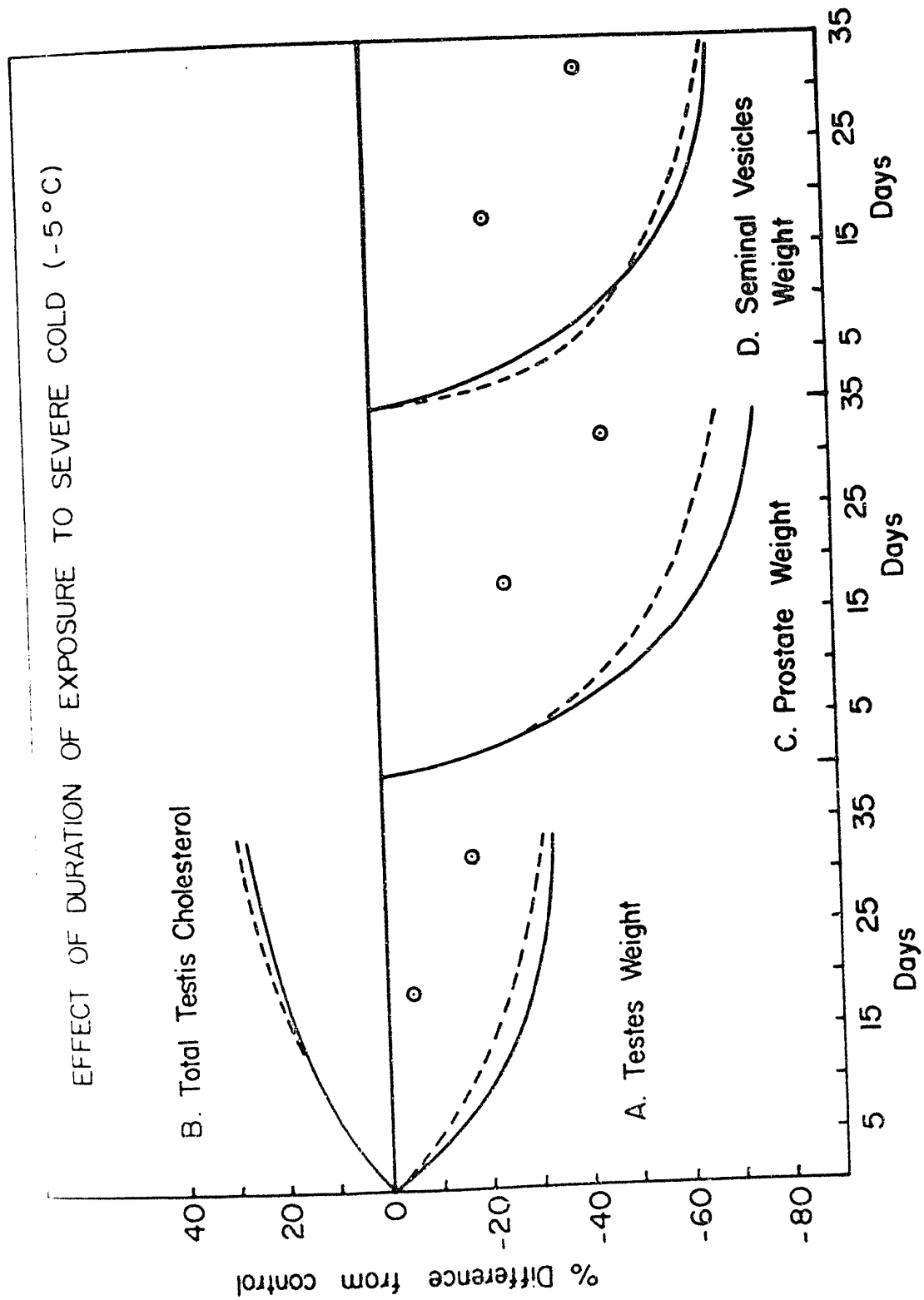


Figure 5, 3.

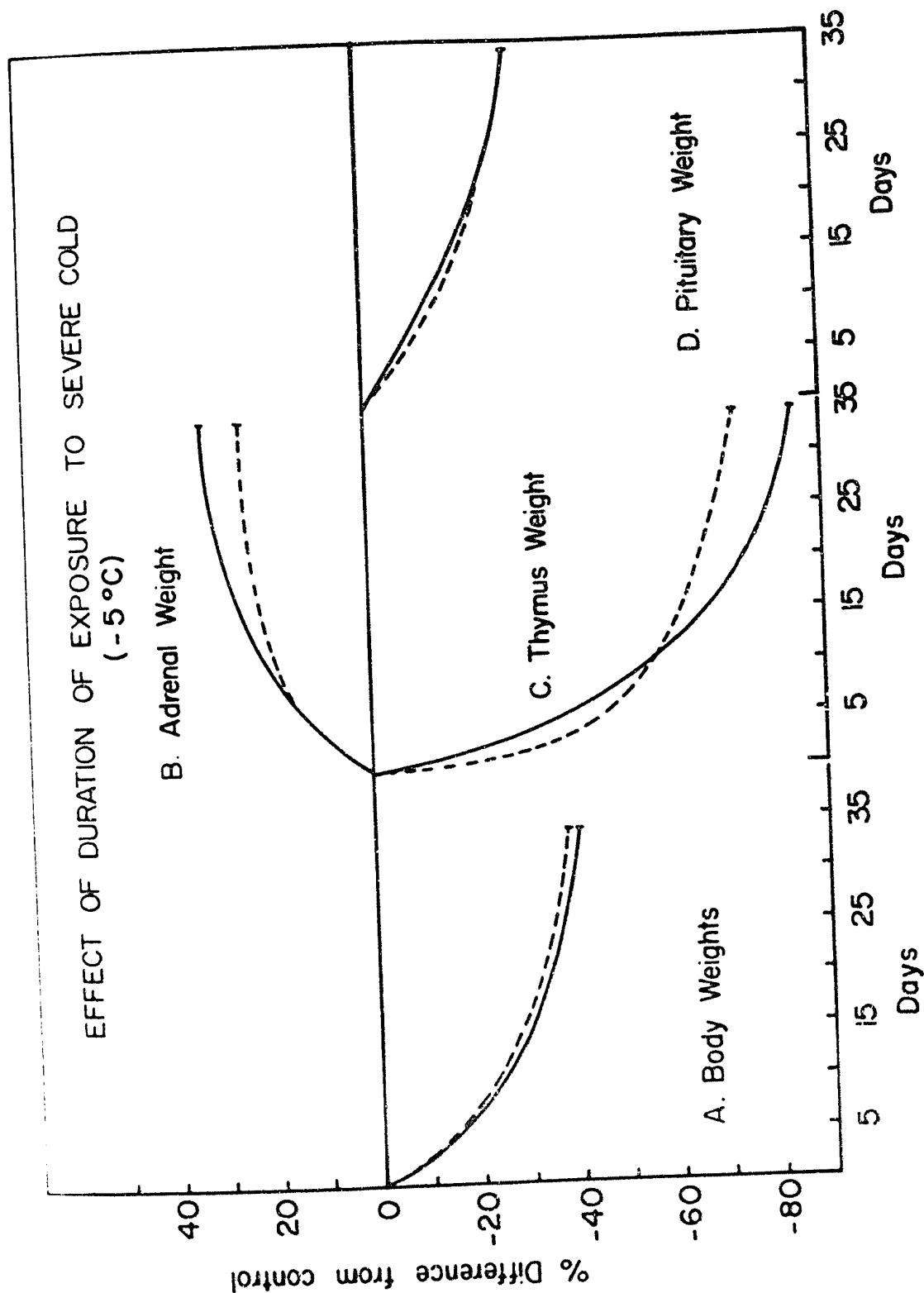


Figure 5, 4.

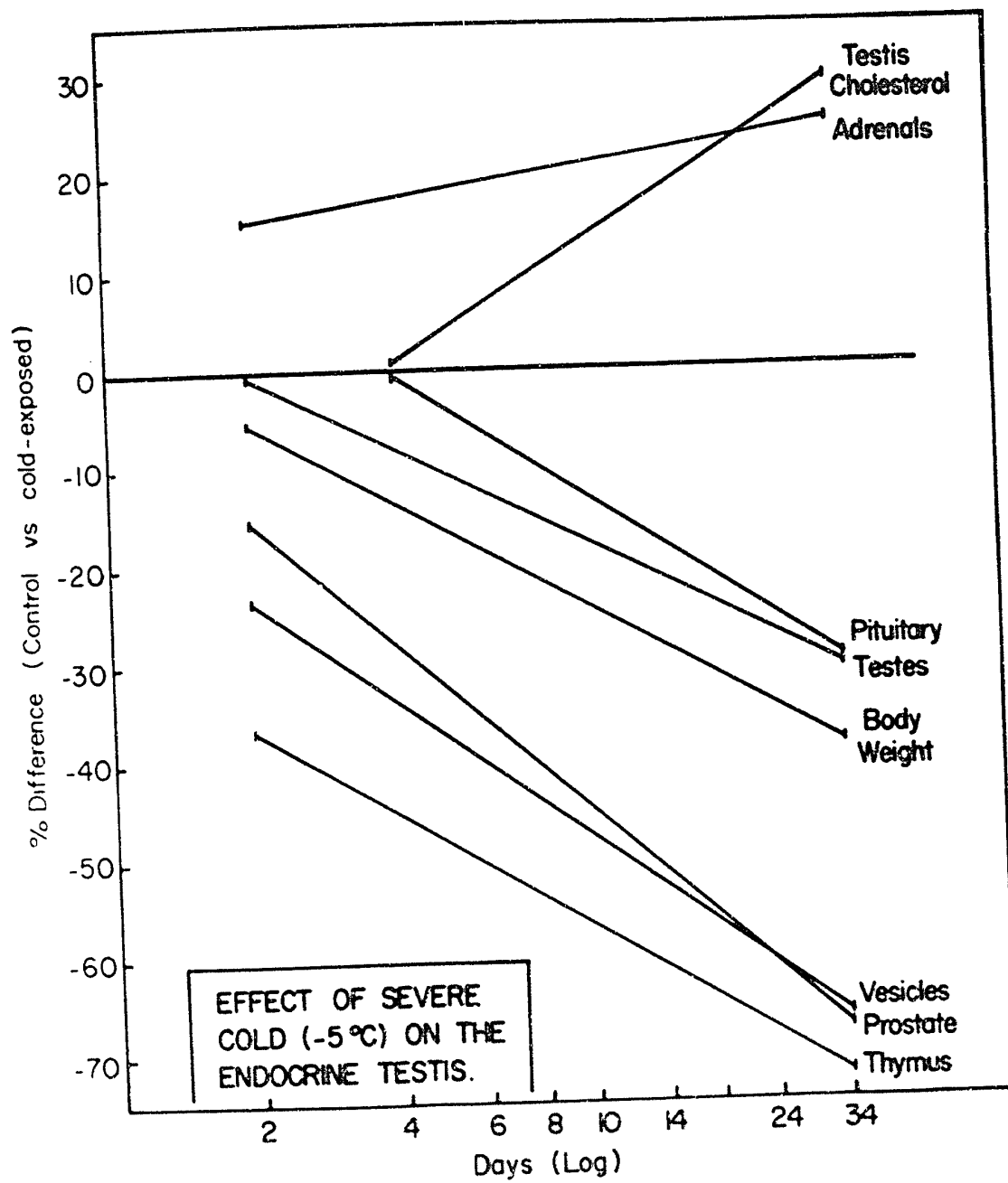


Plate I. Effect of the duration of exposure to severe cold on the testis of the adult rat (125 X - Mallory trichrome).

Figure 1. 5 days - control at room temperature (20°C).

Figure 2. 5 days - exposed to cold (-5°C).

Figure 3. 10 days - control (20°C).

Figure 4. 10 days - exposed to cold (-5°C).

Figure 5. 15 days - control (20°C).

Figure 6. 15 days - exposed to cold (-5°C).

Plate 5, I.

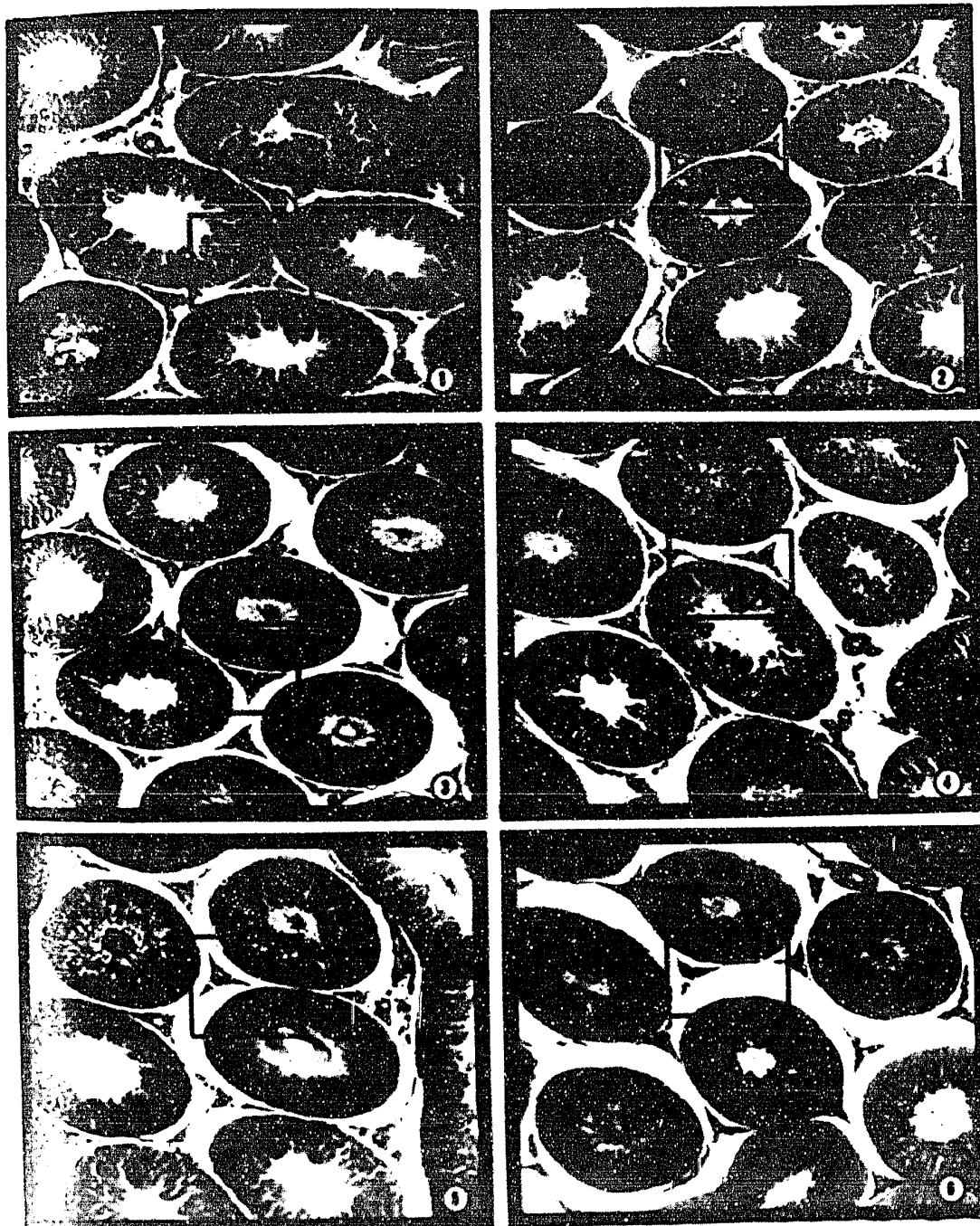


Plate II. Effect of the duration of exposure to severe cold on the testis of the adult rat (125 X - Mallory trichrome).

Figure 7. 20 days - control at room temperature (20°C).

Figure 8. 20 days - exposed to cold (-5°C).

Figure 9. 25 days - control (20°C).

Figure 10. 25 days - exposed to cold (-5°C).

Figure 11. 30 days - control (20°C).

Figure 12. 30 days - exposed to cold (-5°C).

Plate 5, II.

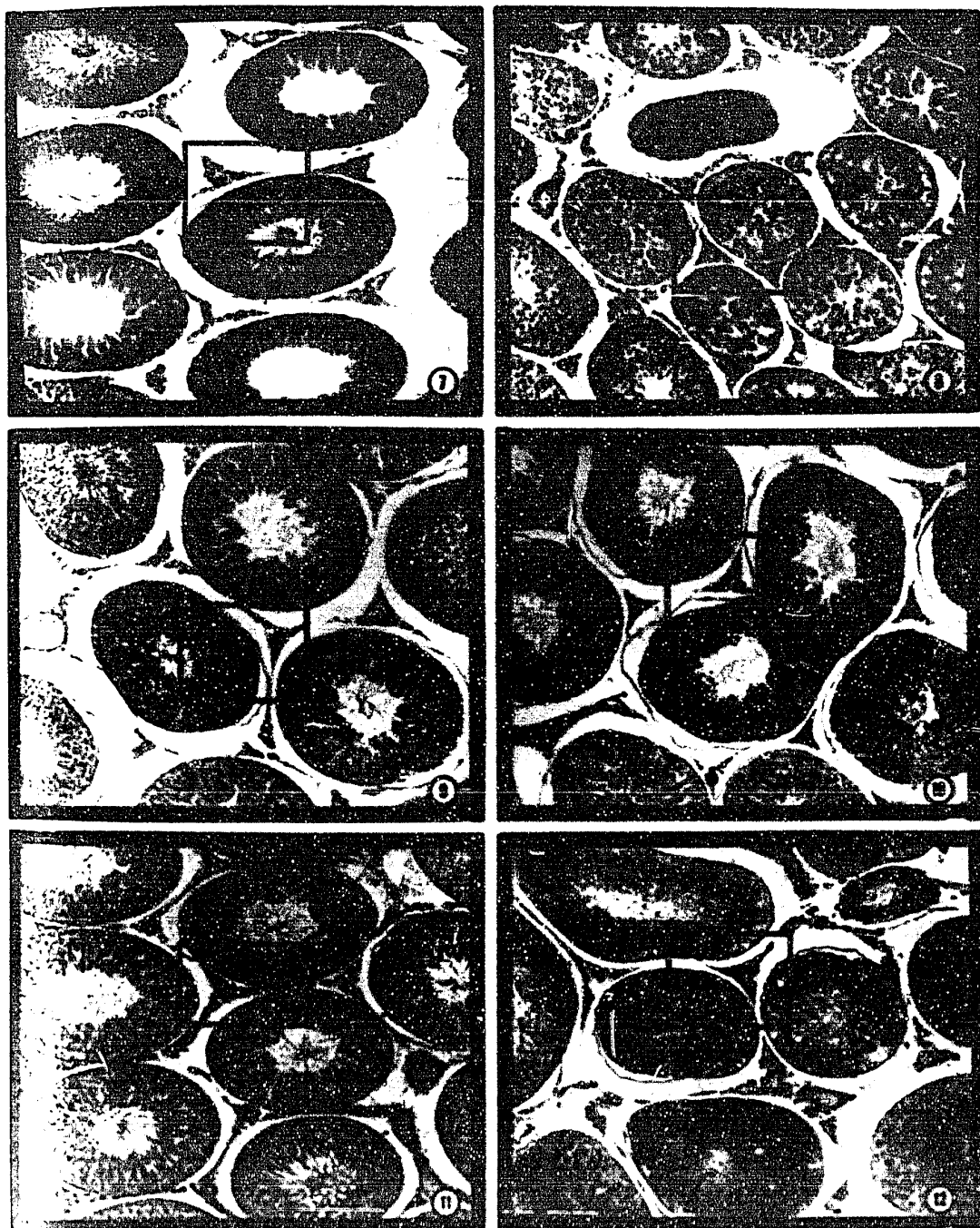


Plate III. Effect of the duration of exposure to severe cold on the testis of the adult rat (500 X - Mallory trichrome).

Figure 13. 5 days - control at room temperature (20°C).

Figure 14. 5 days - exposed to cold (-5°C).

Figure 15. 10 days - control (20°C).

Figure 16. 10 days - exposed to cold (-5°C).

Figure 17. 15 days - control (20°C).

Figure 18. 15 days - exposed to cold (-5°C).

Plate 5, III.

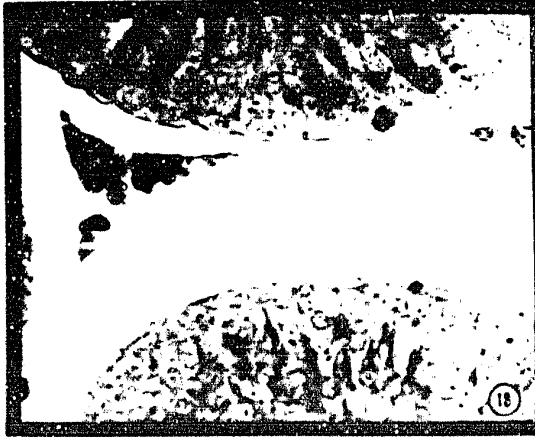
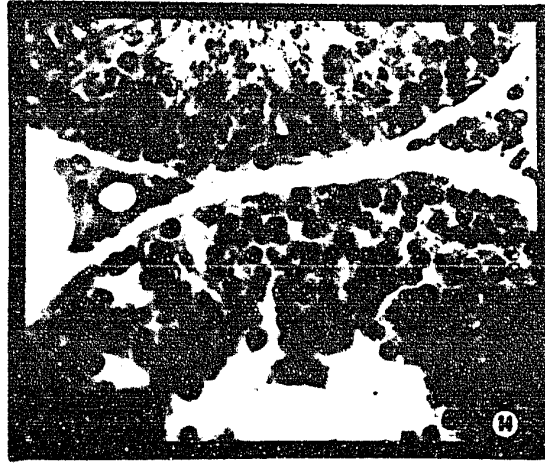
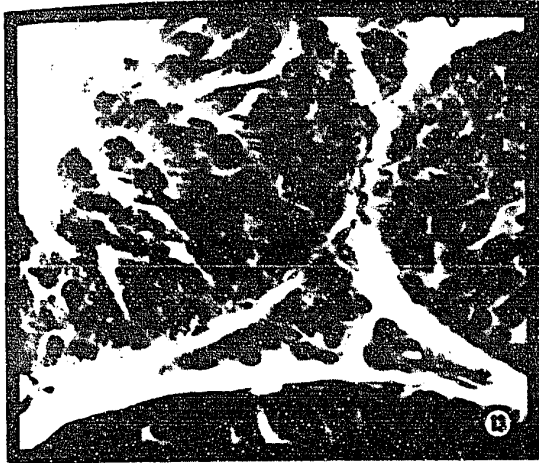


Plate IV. Effect of the duration of exposure to severe cold on the testis of the adult rat (500 X - Mallory trichrome).

Figure 19. 20 days - control at room temperature (20°C).

Figure 20. 20 days - exposed to cold (-5°C).

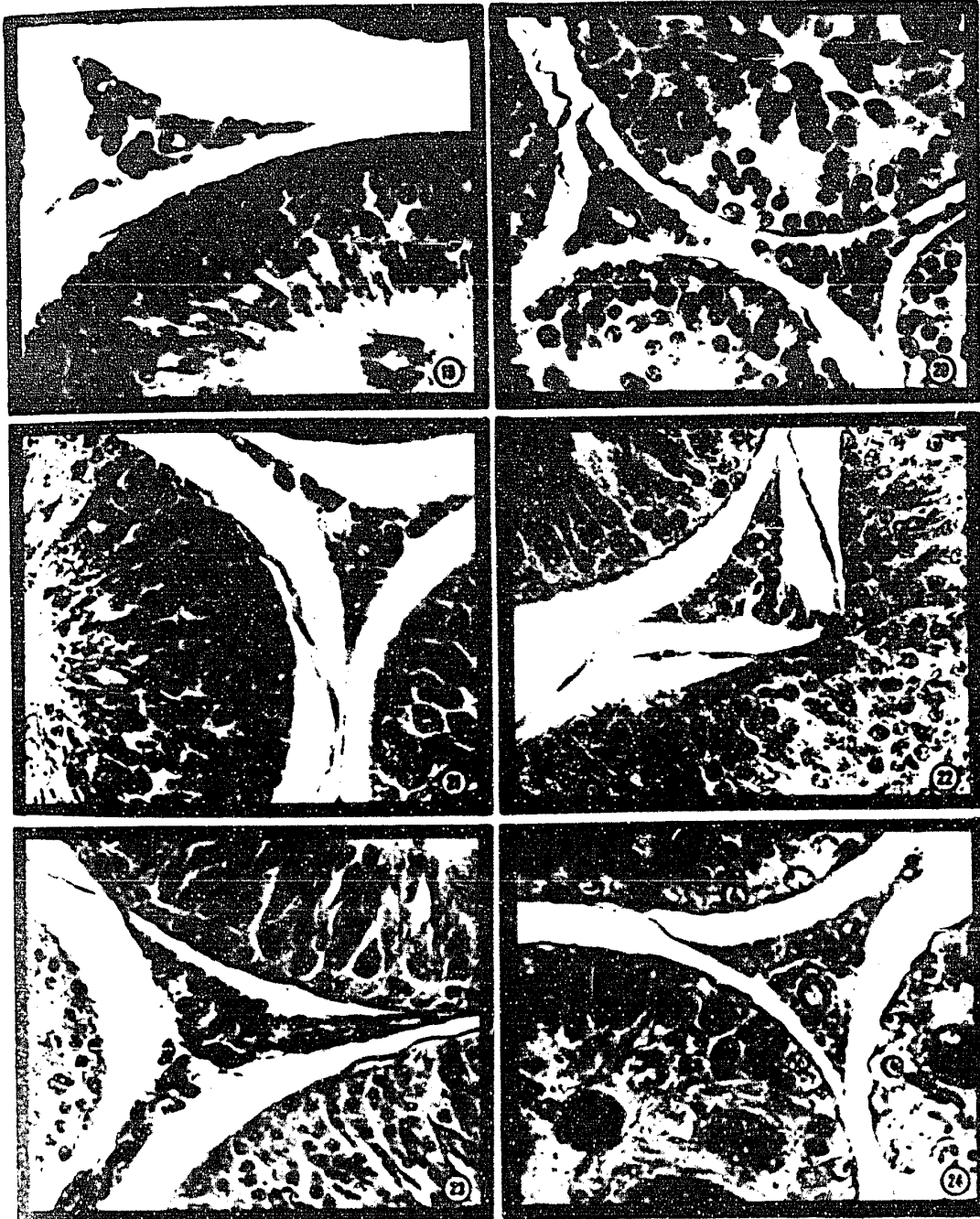
Figure 21. 25 days - control (20°C).

Figure 22. 25 days - exposed to cold (-5°C).

Figure 23. 30 days - control (20°C).

Figure 24. 30 days - exposed to cold (-5°C).

Plate 5, IV.



Chapter Six

THE SOMATIC EFFECTS OF COLD AND THE ENDOCRINE TESTIS

In order to evaluate the significance of the weight changes in the testicular apparatus as a criterion of function, the following assumption was made. The alterations concomitant with general emaciation and consequent to cold exposure may be due to two different sets of conditions:

1) somatic, representing changes associated with nutritional factors which result in metabolic imbalance and eventually inanition.

2) endocrine, representing changes in function not essentially associated with the overall economy and therefore ascribable to cold per se. In other words, if the dietary intake was the only variable, a study of the modifications in the testicular apparatus associated with the level of nutrition, could perhaps be made; thus the pure somatic component of the testicular degeneration could be expressed as the "body weight - organ weight" relationship which obtains in the process of growth and maintenance. This view was strengthened by the occasional observation of signs of typical avitaminoses as part of the cold-injury syndrome. Attractive as it was, this assumption was evidently oversimplified, as purely nutritional states may cause distinct endocrine changes.

A protein-free diet in sexually immature rats results in regression of the reproductive tract and prevents the onset of spermatogenesis; this effect is reversed by protein feeding, but spermatogenic maturation and androgenic release do not follow the same pattern of regeneration, as the accessories recover only partially (1).

On the other hand, hormone action on body growth is a recognized fact: testosterone propionate above physiological level and over a prolonged period, inhibits body weight gains and affects diversely the food intake (2). Adrenalectomized rats, even supported by saline, fail to grow, presumably because of a reduced food intake rather than because of a depressed STH contents in the pituitary; the growth is restored by corticosterone and DOCA (3). The adrenals in thiamine-deficient mice display hypertrophy and respond to ACTH, but not to acute exposure to severe cold (4). Body growth is known to be dependent upon vitamin action with limited influence of the hormones; however, the development of the genital apparatus seems to require a synergism, where the hormones loose effectiveness in the absence of the vitamins (5).

Referring specifically to genital development and fertility, reduced dietary intake and specific protein deficiency have been shown to retard prostate development, even in the presence of a daily dose of 2 mg of testosterone (6); this confirms the observation that dietary restriction or nutritional deficiencies do affect normal fertility (7). The depression of spermatogenesis during and after a severe allergic reaction, associated with antigen-antibody complex, shows a non-specific response of the testes to various traumatic states (8). Ethionine damage to the interstitial tissue is antagonized by testosterone, pointing to an endo-morphological response (9, 10). A cytological picture of hypofunction is seen in the pituitary accompanied by typical atrophy of testes and accessories, in the absence of dietary

protein (11).

Extensive studies of the effect of vitamin deficiencies (12) on the evolution of the sex organs yielded significant information on the separation between the direct effect of the deficiency and the unspecific effect of chronic inanition on the two functions of the genital apparatus (13, 14, 15). Indeed, any action on the sex apparatus may imply (separately or simultaneously, with various degrees of potentiation or interference) metabolic alterations, reduction in the synthesis of sex hormones, disturbance in the hypothyseal relay and interference with the hepatic metabolization of steroids; thus, the sex apparatus has been shown to be sensitive to nutritional dysbalance as well as to specific avitaminoses (16, 17, 18, 19).

The latter studies were conducted under the following experimental design: three groups were formed: 1) ad libitum feeding, 2) deficient diet, 3) diet complete in all factors, but equal in amount and caloric value with the actual quantities ingested by the deficient animals. This experimental arrangement inspired our own design: a reduced intake of a balanced diet would permit adjustment of the body weights to the rate and level of loss typical of the cold-exposed animals; by maintaining this level of body weight during a period comparable to the duration of the exposure to cold, it might become possible to relate the effects observed in the testicular apparatus to purely somatic conditions.

MATERIALS AND METHODS

A group of male rats (Wistar Albino) was arranged according to the norm of Table I.

Table I

Distribution of animals				
		Normal diet (N)	Restricted Diet (R)	
			18 days	32 days
Absolute controls	(O)	NO (14) ¹	RO (30)	---
Sham-operated	(A)	NA (10)	---	RA (10)
Treated castrates	(B)	NB (10)	---	RB (15)
Untreated castrates	(C)	NC (10)	---	RC (16)

¹: Group population

After the usual 4 to 5-day quarantine, the animals were grouped and "starvation" began. Just enough chow pellets were made available to induce a loss of body weight at a rate similar to that observed in the cold; maintenance of the body weight at the desired level was obtained by empirically adjusting the amount of food to the daily weighings.

On days 7 and 8, 51 animals were castrated by abdominal approach and 20 were sham-operated; two days of normal feeding were allowed

to help recovery before resumption of the restricted diet. The absolute controls were introduced later, so that their terminal body weight would be similar to that of the normally fed subjects (Figure 1). Testosterone treatment consisted of a single intramuscular injection of 2 mg of Perandren Cristoules (Testosterone Propionate - Giba) in 0.5cc of saline, administered 7 days after surgery. Purina lab chow (pellets) was offered ad libitum to the "Normal Diet" groups and controlled (as described above) to the "Restricted Diet" group; tap water was ad libitum for all groups. The animals were caged individually in air-conditioned quarters maintained at 20 - 22°C and illuminated naturally; the procedure was terminated after the durations indicated: 18 days and 32 days of restricted feeding.

RESULTS

Body weights (Table II)

The intent of the procedure was to restrict the food intake and obtain a reduction in body weights, at a rate and level approximately equal to the values observed in the cold-exposed animals; Table II and Figure 1 show that the attempt proved successful. No difference, due to castration and/or therapy is observable, showing that the weight control was fully effective; Figure 1 also shows that the calculated synchronization of the absolute controls was correct.

Table II

Body weights (grams)			
[Prior to diet restriction: 265.4 ± 2.0 (115)]			
Groups	Pre-surgical	Terminal	Duration
Absolute controls			
Normal diet (NO)	----	335.1 ± 4.8 (14)	18 days
Restricted diet (RO)	----	237.3 ± 1.0 (30) <u>-29%</u> (44)	
Sham-operated			
Normal diet (NA)	291.0 ± 5.8 ¹ (10) ²	349.1 ± 8.5 (9)	32 days
Restricted diet (RA)	244.9 ± 3.3 (10) -15% (20) ³	228.4 ± 8.9 (9) <u>-34%</u> (18)	
Treated castrates			
Normal diet (NB)	290.9 ± 5.2 (10)	344.4 ± 8.3 (9)	32 days
Restricted diet (RB)	245.3 ± 3.2 (15) -15% (25)	224.5 ± 5.3 (15) <u>-35%</u> (24)	
Untreated castrates			
Normal diet (NC)	289.9 ± 5.2 (10)	332.5 ± 11.0 (10)	32 days
Restricted diet (RC)	243.9 ± 3.3 (16) -15% (26)	228.6 ± 4.4 (16) <u>-31%</u> (26)	

- 1: Standard error of the mean. —: $p < 0.001$
 2: Group population
 3: Aggregate population

Analysis of variance - summary

	Effect of treatment	Effect of diet restriction	Interaction
Pre-surgical body weights	N.S.	$t = 12.7$ $p < 0.001$	N.S.
Terminal body weights	N.S.	$t = 14.4$ $p < 0.001$	N.S.

Organ weightsTestes (Table III)

The observed weight depression displays a difference ascribable to dietary restriction; although not significant and within the range of physiological variation, this difference due to time (-8%) proves interesting (Control - 18 days versus Sham - 32 days).

Table III

Testes (weight in grams)				
Groups	Normal diet (N)	Restricted diet (R)		Difference
		18 days	32 days	
Absolute controls (O)	$3.28 \pm 0.08^1 (14)^2$	$3.15 \pm 0.05 (30)$	----	- 5% (44) ³
Sham-operated (A)	$3.49 \pm 0.09 (9)$	----	$2.89 \pm 0.08 (8)$	-17% (17)

1: Standard error of the mean.

----: $p < 0.001$

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of surgery	1	0.0112	---	0.14	N.S.
Effect of diet restriction	1	1.5212	---	19.33	* *
Interaction	1	0.9202	---	11.69	* *
Error	58	4.566	0.0787		

Effect of diet restriction: $t = 4.64$ $p < 0.001$

Sexual accessories (Tables IV and V)

The effect of castration and of therapeutic replacement buffers the overall evaluation of the effect of dietary restriction; however, the sensitivity of the accessories displays a fine discriminating response to the level of androgenic factors. Specifically, the testosterone treatment obliterates completely the effect of reduced dietary intake which shows markedly among the untreated castrates.

The effect of diet restriction is remarkably uniform between prostate and vesicles and associated with duration of the starvation diet.

A curious sparing effect appears in the prostate of the castrate (RB) under starvation; granted that the prostate is more responsive than the vesicles to the titer of circulating androgens, this "improvement" of the prostate and of the vesicles (to a lesser degree) in the starved treated castrate proved unexpected.

Table IV

Prostate
(weight in grams)

Groups	Normal diet (N)	Restricted diet (R)		Difference
		18 days	32 days	
Absolute controls	(O) 0.44 ± 0.02^1 (14) ²	0.33 ± 0.03 (30)	----	<u>-25%</u> (44) ³
Sham-operated	(A) 0.60 ± 0.03 (9)	----	0.33 ± 0.03 (9)	<u>-45%</u> (18)
Treated castrates	(B) 0.17 ± 0.02 (9)	----	0.30 ± 0.04 (15)	<u>+43%</u> (24)
Untreated castrates	(C) 0.08 ± 0.009 (10)	----	0.06 ± 0.006 (16)	-25% (26)

1: Standard error of the mean.

—: $p < 0.01$

2: Group population.

3: Aggregate population.

==: $p < 0.001$

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	3	2.077	0.692	11.94	**
Effect of diet restriction	1	0.086	—	14.89	**
Interaction	3	0.435	0.145	24.98	**
Error	105	0.607	0.006		

Effect of treatment: O A B CEffect of diet restriction: $t = 1.89$ $p > 0.05$ N.S.

Table V

Seminal vesicles (weight in grams)					
Groups	Normal diet (N)	Restricted diet (R)		Difference	
		18 days	32 days		
Absolute control	(O) 0.57 ± 0.02^1 (14) ²	0.44 ± 0.01 (30)	---	-23%	(14) ³
Sham-operated	(A) 0.60 ± 0.02 (9)	---	0.35 ± 0.02 (9)	-42%	(18)
Treated castrates	(B) 0.25 ± 0.01 (9)	---	0.31 ± 0.03 (15)	+19%	(24)
Untreated castrates	(C) 0.13 ± 0.01 (10)	---	0.11 ± 0.01 (16)	-15%	(26)

1: Standard error of the mean.

---: $p < 0.05$

2: Group population.

---: $p < 0.01$

3: Aggregate population.

---: $p < 0.001$

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	3	2.569	0.856	171.3	**
Effect of diet restriction	1	0.195	--	39.0	**
Interaction	3	0.156	0.052	10.4	**
Error	104	0.520	0.005		

Effect of treatment: O A B C

Effect of diet restriction: $t = 1.16$ $p > 0.20$ N.S.

Adrenals (Table VI)

The feminization effect of castration is reversed by testosterone treatment among the normally fed animals; dietary restriction cancels the hypertrophy in the castrate and prevents any testosterone effect.

The overall adrenal response shows that: 1) surgery proved in no way stressful, and 2) dietary restriction decreases the adrenal weight instead of inducing a stress response of hypertrophy.

Table VI

Adrenal glands
(weight in milligrams)

Groups	Normal diet (N)	Restricted diet (R)		Difference
		18 days	32 days	
Absolute controls (O)	52.5 ± 1.6 ¹ (14) ²	44.4 ± 1.8 (30)	-----	-15% (44) ³
Sham-operated (A)	50.8 ± 2.1 (9)	-----	39.9 ± 1.8 (9)	-21% (18)
Treated castrates (B)	52.2 ± 1.6 (9)	-----	42.5 ± 1.9 (15)	-18% (24)
Untreated castrates (C)	60.0 ± 3.5 (10)	-----	42.8 ± 0.8 (13)	-29% (23)

1: Standard error of the mean.

--: p < 0.05

2: Group population

---: p < 0.01

3: Aggregate population

----: p < 0.001

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	3	431.8	143.9	2.34	N.S.
Effect of diet restriction	1	3404.2	--	55.4	* *
Interaction	3	378.6	126.2	2.1	N.S.
Error	101	6203.7	61.4		

Effect of diet restriction: t = 6.90 p < 0.001

DISCUSSION

1) Effect of physiological condition.

Once more, we observe that the body weight is not visibly affected by the physiological status created by castration with or without treatment. The changes in the sex accessories due to castration are well known and the effect of a low grade of replacement therapy proves effective in bringing these accessories to some minimal level of regeneration.

2) Effect of dietary restriction.

The initial assumption implied that if a value of body weight equivalent in rate and in level to that observed after cold exposure was obtained, the magnitude of the "somatic" effects of cold could perhaps be evaluated; we like to think that dietary restriction renders possible a discussion of this aspect of the response to cold. Some remarks are suggested by the present observations:

1. Two periods of starvation were selected to conform with time intervals already utilized; holding back the body weight and the growth process simulates the situation caused by cold where duration of the stress induces effects of similar amplitude.

2. The loss of weight in any of the measured organs seems to be characteristic of that organ, proportional but not equal to the loss of body weight.

3. The dichotomy "control-experimental" cancels out the strain or season effect and places the subjects on a direct comparative basis.

4. The situation created by dietary restriction does not elicit in the adrenal a stress response; one could infer that the adrenal is not to a significant level, engaged endocrinologically. However, the cancellation by dietary restriction of the feminization of the adrenal in the castrate, although not statistically significant, is disturbing.

5. The sex accessories respond to the physiological status of castration (with or without treatment) in the typical manner: that of a secondary sex organ and a functional indicator of activity in the primary organ; this response is unaffected by dietary restriction within the starved groups. However, in the ^{treated} castrate an unexpected elevation of accessory weight (not significant, but amazingly uniform) appears due to dietary restriction. Is this difference caused by the anabolic action of therapeutic testosterone and resulting in a "sparing" effect? Is there an influence of adrenal androgens on the accessories?

The second possibility seems unlikely; indeed, the adrenals would appear rather hypofunctional, the situation being unstressful and the feminization of the gland absent. Even admitting that the threshold of the prostatic response to circulating androgens is lower than that of the vesicles, the two indicators respond in the same direction pointing to a curious sparing effect of a potent anabolic agent.

In any event, the pattern of weight depression in the testicular apparatus gives a fairly consistent picture: the loss in the primary

organ is moderate, but related to duration of the dietary restriction; the loss in the sexual accessories is in the same order of magnitude as the loss in body weight due to dietary restriction. Furthermore, this response in the sexual accessories is cancelled by application of testosterone, showing that the endocrine reaction is distinct and dissociated from the "somatic" response.

If the weight depressions observed in the present procedure are plotted on the curves given in Chapter 5 (Figure 4) the following balance sheet results:

Table VII

% difference between "Control versus experimental"				
		Restricted diet	Overall effect of cold (from theoretical curves)	Difference (cold minus starvation)
Body weight	18 d.	-29%	-32%	----
	32 d.	-34%	-38%	----
Testes weight	18 d.	- 5%	-24%	-19%
	32 d.	-17%	-30%	-13%
Prostate wt.	18 d.	-25%	-56%	-31%
	32 d.	-45%	-67%	-22%
Vesicles wt.	18 d.	-23%	-57%	-35%
	32 d.	-42%	-66%	-22%
Adrenal wt.	18 d.	-15%	+23%	----
	32 d.	-21%	+25%	----

If now the initial assumption is resumed, these results could be read as follows: the effect of the restricted diet represents the so-called purely somatic response of the endocrine organs, i.e. the dependence of these organs on the general health and economy of the organism. Under conditions of starvation, the overall balance operates at a lower (and more economical) level and thus becomes responsible for a general depression of the labile constituents of all body parts; in one word, this association represents a direct proportionality between body weight (and age) and organ weight. Thus, starvation would represent conditions obtaining at the chronological age corresponding to the observed body weight.

Under exposure to cold stress, a degeneration occurs which evidently engages the structural components of the body and all its parts, but this response goes further in that certain organs reflecting the functional level of a primary organ are depressed far beyond the purely somatic response and thus, display proof certain of an endocrinological dysfunction as part of the overall response. Reading from Table VII, one would be inclined to interpret the "differences" as representing the endocrine fraction of the stress response.

It goes almost without saying that this view is strictly provisional and should not lead to sanguine conclusions. A number of reservations could be made; nothing has been said about the effect of malnutrition (by imbalance or insufficient intake) leading to avitaminoses and secondarily to anorexia and eventually inanition,

resulting in the so-called "pseudo-hypophysectomy" (20) (in the present case, the adrenal response may be due to this situation). Furthermore, the lipid metabolism is modified (at least in terms of rate) by reduced food intake (21, 22, 23); lastly, the recognized impairment of glucose utilization resulting from hypothermia may well cause significant disturbances in the testicular physiology (24, 25). Other events such as modification of thyroid function and the overall picture of oxydo-reduction reactions may well affect the evolution of the testicular apparatus (26, 27).

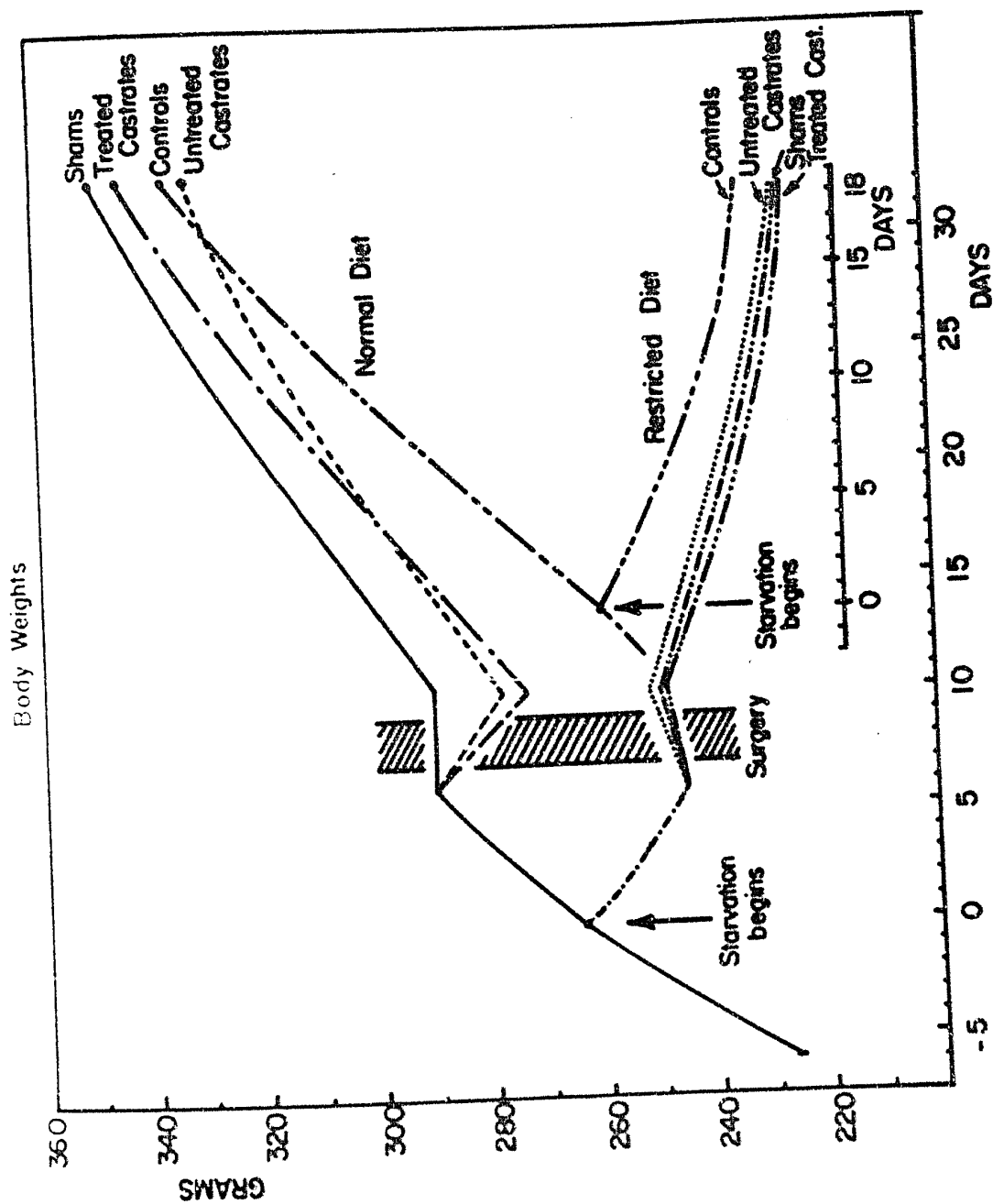
These reservations invite caution but the basic distinction of 2 components in the overall effect of cold on the testicular function remains valid, i.e. the distinction between a somatic component (of structural nature) and a strictly endocrine response, perhaps involving the axis "hypothalamus - pituitary - testis-accessories" and resulting in a severe depression of androgenic activity.

Therefore, assuming that the overall damage represents an anatomical lesion resulting from metabolic disturbances and from endocrine impairment, one could state that the morphological damage approaches a limit defined by the structural elements of the organs and that the functional lesion approaches a limit defined by endocrine homeostasis (slowing down, arrest or switch) in the functional pattern.

CONCLUSIONS

1. Dietary restriction simulates truly the somatic depression due to cold, but imposes no stress on the organism;
2. The "body weight-organ weight" relationship is not linear and uniform; the mathematical expression is characteristic of the organ concerned;
3. The dichotomy "control-experimental" of same initial age, cancels out strain and season effects and places the subjects on a strictly comparative basis;
4. The endocrine response of the sex accessories is distinct from and unaffected by the somatic changes; therapy evokes an accessory response in direct proportion to dosage;
5. The weight changes in the primary endocrine organ (e.g. testis) is at the same level as the loss in body weight; the primary organ is sensitive to somatic variations, but these alterations are not reliably informative of functional changes;
6. The testicular response to cold is made of two components: somatic and endocrine, each progressing at a typical rate and approaching a particular asymptotic limit.
7. Judgment should be reserved on the "non-response" of the adrenal in the diet-restricted animal. "Starvation" may well suppress a potential stress response: a reduced ACTH production (pseudo-hypophysectomy) failing to stimulate the adrenal, otherwise impaired by the reduced food intake.

Figure 6, 1.



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Chapter Seven

DISSOCIATION OF THE EFFECT OF SEVERE COLD (-5°C)
ON THE TESTIS, INTO SOMATIC AND ENDOCRINE COMPONENTS

Repeated observations have shown that in the cold-exposed rat, the testes undergo a distinct degeneration. In a chronic exposure to moderate cold (2°C), this degeneration appears at approximately 40 weeks as a loss of function, exocrine and endocrine as well, along with morphological alterations, such as changes in weight, modifications of the histological picture of the tubules, etc. (1). In acute exposure to severe cold (-5°C), the same process becomes observable within a period of 11 days; the testes show considerable decrease in weight, and the sexual accessories, prostate and seminal vesicles, possess the typical eunuchoid characteristics (2).

One common feature is the position of the testes in the abdominal cavity; indeed, the testes migrate reflexly into the abdomen shortly after the beginning of the cold exposure. This could be interpreted as the cremasteric reflex which occurs in response to a number of stimuli, for example, hot or cold applied to the tail and mechanical stimulation of the hind quarters. The testes remain abdominal as long as the cold stress is applied, and at postmortem, the animal may be described as cryptorchid.

The effects of cryptorchism on spermatogenesis (3) and on the sexual behaviour (4) are firmly documented; the reports on the effects of cryptorchism on the androgenic function of the testis are less uniform. A slow (observable at 1 year post-operatively) impairment of androgenic activity is noted in the young animals, but not in the older subjects (5). Biotin deficiency associated with egg-white injury in weanling rats, prevents testicular descent, while in

30-day old rats, the deficient diet brings about movement to the abdominal position; the assigned reason: contracture of the cremaster muscle (6). At 4 months post-operatively, the cryptorchid testis displays severe germinal damage, but intact Sertoli cells and interstitium (7). However, assays have shown that the ability of the testis to convert progesterone to known terminal steroids, was markedly reduced in the cryptorchid testis even below the level of the testis in the hypophysectomized animal; assigned reason: decreased enzymatic activity (8).

At this point, two questions arise: 1) Is the testicular degeneration (in structure and in function) consequent upon cold exposure due primarily to cryptorchism or to cold per se? 2) Is it possible to dissociate experimentally the effect of cold from the effect of cryptorchism?

MATERIALS AND METHODS

Animal groups.

A random group of 182 male rats (Wistar Albino) was arranged as shown in Table I and surgical manipulations applied accordingly.

Table I

Distribution of animals			
Description		20°C (N)	-5°C (F)
Absolute controls	(A)	AN (13) ¹	AF (18)
Sham-operated	(B)	BN (11)	BF (16)
Right testis abdominal	(C)	CN (11)	CF (17)
Both testes abdominal	(D)	DN (16)	DF (32)
Both testes scrotal	(E)	EN (17)	EF (31)

1: Group population

Surgery.

The procedure consisted in placing the testes in a fixed position, thus creating in some groups irreversible cryptorchism, unilateral or bilateral, and in others, preventing migration of the testes (9). The absolute controls (group A) were not subjected to surgery, but were assigned a 5-day quarantine period before entry into the cold. All operative manipulations, except ligation of the inguinal canal, were applied to the shams.

Living quarters and diet

Prior to cold exposure, the animals lived in air-conditioned quarters maintained at approximately 20°C and were fed Purina Lab Chow and tap water ad libitum. All animals entered the cold simultaneously on day Zero and were exposed to a temperature of -5°C $\pm$ 1 for 25 days; the rooms were ventilated, thermostatically controlled, and illuminated under automatic control, from 9.00 a.m. to 5.00 p.m., daily.

Autopsy data.

Postmortems were performed on all animals within a 2-day period; organs were collected, examined, and weighed wet. Specimens of prostates were taken for microscopic examination; tissues were fixed in Bouin fluid, paraffin-embedded, sectioned at 5 μ , and stained with Mallory trichrome. The examination was strictly histological; no attempt was made at cytological findings, histochemical evaluation, or quantitative measurements of cell size, population, or association.

Statistics.

All values were screened by means of the Dixon test for extreme values (10), resulting in a very small number of rejected extremes; the analysis of variance was performed according to the "R x 2 tables" of Yates (11) complemented by the Students' T and/or the "multiple range test" of Duncan (12). All tables quote the arithmetical mean with the standard error of the mean.

RESULTS

1. Body Weights (Table II)

The preoperation body weights represent the values immediately prior to surgery and the preexposure body weights, the values immediately prior to entry into the cold room. The animals were selected with the minimum possible difference in body weights; the statistical test shows that the attempt proved successful. The terminal body weights demonstrate the effect of cold only and illustrate the innocuous character of the surgery performed and of the resulting physiological status.

Table II

Body weights (grams)				
Groups	Preoperation	Preexposure	Terminal	Difference
Absolute controls	(AN) (AF)	270.5 ± 2.3 (13) 269.4 ± 2.2 (18)	354.8 ± 6.2 (12) 248.1 ± 4.5 (13)	-30% (25) ³
Sham-operated	(BN) (BF)	244.5 ± 3.1 ¹ (11) ² 242.2 ± 3.3 (16)	350.2 ± 8.7 (9) 248.5 ± 5.9 (15)	-29% (24)
Partial cryptorchism	(CN) (CF)	246.8 ± 3.2 (11) 243.4 ± 3.6 (17)	336.3 ± 8.2 (11) 253.0 ± 4.9 (10)	-25% (21)
Total cryptorchism	(DN) (DF)	241.1 ± 3.6 (16) 242.8 ± 2.6 (32)	331.3 ± 6.3 (15) 236.4 ± 5.0 (24)	-29% (39)
Scrotal position	(EN) (EF)	242.1 ± 3.5 (17) 243.2 ± 2.3 (31)	337.4 ± 9.1 (15) 242.9 ± 5.2 (26)	-28% (41)

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

==: p < 0.001

Analysis of variance - summary

	Effect of treatment	Effect of cold	Interaction
Preoperation body weights	N.S.	N.S.	N.S.
Preexposure body weights	N.S.	N.S.	N.S.
Terminal body weights	N.S.	t = 22.04 p < 0.001	N.S.

The growth curves (Figure 1) smoothed out in free hand, tell the dynamic story of the changes due to cold and support the statistical data of Table II: cold alone induces uniformly the alterations observed in the growth process.

2. Survival rates (Table III).

The survival rates through the 25 experimental days display a normal scattering of values and, due to the small size of the group populations, do not warrant any valid surmise on the relationship between survival and the physiological condition of the various groups.

Table III

Survival rates of cold-exposed subjects (% of initial group population)								
Groups	Days	0	4	8	12	16	20	25
Absolute controls	(AF)	100 (18) ¹	100	100	100	100	83	72 (13) ²
Sham-operated	(BF)	100 (15)	100	100	100	100	100	100 (15)
Partial cryptorchism	(CF)	100 (16)	100	100	88	88	75	62 (10)
Total cryptorchism	(DF)	100 (30)	97	96	96	93	93	80 (24)
Scrotal position	(EF)	100 (30)	100	97	97	97	93	87 (26)

¹: Initial population at exposure time.

²: Terminal population at autopsy.

3. Organ weights.

1) Testes.

In gross appearance, the cryptorchid testis at room temperature is bluish, very small in size, and of very low turgidity; the cold-exposed testis, soft and reduced in size, appears pale and devoid of the congestion observed (13,14) in the cryptorchid testis at room temperature.

Inspection of Table IV along with the analysis of variance suggests the following remarks. A significant difference due to cold appears in all exposed groups (except the sham-operated); cryptorchism, however, marks its own boundaries of significance: between non-cryptorchid versus cryptorchid and between partial (C) versus total cryptorchid (D). Cold and cryptorchism appear to act in the same direction, but independently and disjunctively; thus cold per se produces its effect irrespective of the "position" of the testis.

The shams present a curious picture: slight depression of the testis weight at room temperature (-9% relative to the Control) and notable sparing in the cold (7% relative to the Control). The divergence precludes an effect of the surgical manipulations and remains without explanation.

Table IV

		Testes (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	3.37 ± 0.09 ¹ (12) ²	2.82 ± 0.10 (13)	- <u>16%</u> (25) ³
Sham-operated	(B)	3.08 ± 0.06 (8)	3.02 ± 0.06 (14)	- 2% (22)
Partial cryptorchism	(C)	2.44 ± 0.06 (5)	1.81 ± 0.05 (6)	- <u>25%</u> (11)
Total cryptorchism	(D)	1.45 ± 0.06 (14)	1.04 ± 0.03 (23)	- <u>25%</u> (37)
Scrotal position	(E)	3.01 ± 0.10 (14)	2.50 ± 0.06 (15)	- <u>17%</u> (29)

1: Standard error of the mean.

—: p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	4	743.08	185.77	210.15	**
Effect of cold	1	8.8125	8.8125	9.97	**
Interaction	4	0.9446	0.2362	0.27	N.S.
Error	104	91.90	0.884		
Effect of treatment:	A	B	E	C	D
Effect of cold:	t=3.40 p < 0.001				

These remarks are substantiated by Tables V and VI, where the data from the right and the left testis are dissociated. In groups CN-CF and DN-DF where the right testis is cryptorchidic, cryptorchism is the only cause of significance, but the order of values reveals the effect of cold in the direction of depression of testis weight, whether the testis is in "normal" position or cryptorchid.

Table V

		Right testis (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	1.73 ± 0.04^1 (12) ²	1.39 ± 0.06 (13)	<u>-19%</u> (25) ³
Sham-operated	(B)	1.55 ± 0.03 (8)	1.53 ± 0.03 (14)	- 1% (22)
Partial cryptorchism	(C)	0.67 ± 0.05 (10)	0.50 ± 0.02 (9)	<u>-25%</u> (19)
Total cryptorchism	(D)	0.67 ± 0.03 (15)	0.54 ± 0.01 (20)	<u>-19%</u> (35)
Scrotal position	(E)	1.62 ± 0.04 (12)	1.29 ± 0.04 (21)	<u>-20%</u> (33)

1: Standard error of the mean.

2: Group error population

3: Aggregate population

—: $p < 0.001$

—: $p < 0.01$

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F obs.	
Effect of treatment	4	26.72	6.68	28.32	* *
Effect of cold	1	1.23	1.23	5.20	*
Interaction	4	0.4614	0.1154	0.49	N.S.
Error	124	29.25	0.2359		
Effect of treatment:	A B E		C D		
Effect of cold:	t = 1.88 p > 0.05				N.S.

When the left testis is cryptorchid (groups DN-DF), the same trend appears as in Table V, although to a lesser degree: cryptorchism marks the boundary of significance, but cold per se follows the expected trend of a specific depression.

Table VI

		Left testis (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	1.69 ± 0.04 ¹ (11) ²	1.43 ± 0.05 (13)	-15% (24) ³
Sham-operated	(B)	1.56 ± 0.04 (9)	1.49 ± 0.03 (14)	-10% (23)
Partial cryptorchism	(C)	1.60 ± 0.02 (7)	1.32 ± 0.06 (7)	-17% (14)
Total cryptorchism	(D)	0.73 ± 0.05 (15)	0.51 ± 0.02 (24)	-30% (39)
Scrotal position	(E)	1.57 ± 0.03 (12)	1.21 ± 0.05 (18)	-18% (30)

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

—: $p < 0.001$

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F obs.	
Effect of treatment	4	19.65	4.91	22.64	**
Effect of cold	1	1.63	1.63	7.53	**
Interaction	4	0.2724	0.0681	0.31	N.S.
Error	120	26.03	0.2169		
Effect of treatment:	A	B	E	C	D
Effect of cold:	t = 3.81 p < 0.001				

ii) Sexual accessories.

The seminal vesicles were uniformly emptied of their contents and weighed wet; the ventral prostates, after removal of the fatty sheath, were weighed and submitted to microscopic examination.

The weight of the accessories shows remarkable consistency, whether in the cold or at room temperature. The only significant difference is due to cold, constantly and specifically: the weights of the prostate (Table VII) and the seminal vesicles (Table VIII) are significantly different at the 1% level.

Cryptorchism leaves intact, at normal temperature and in the cold as well, the characteristic weight level of the accessories.

Table VII

		Prostate (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	0.43 ± 0.02 ¹ (11) ²	0.26 ± 0.02 (13)	-39% (24) ³
Sham-operated	(B)	0.34 ± 0.03 (9)	0.25 ± 0.02 (15)	-26% (24)
Partial cryptorchism	(C)	0.45 ± 0.03 (11)	0.25 ± 0.02 (10)	-44% (21)
Total cryptorchism	(D)	0.43 ± 0.02 (14)	0.24 ± 0.02 (23)	-44% (37)
Scrotal position	(E)	0.45 ± 0.03 (15)	0.27 ± 0.02 (26)	-40% (41)

1: Standard error of the mean.

—: p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	4	0.0532	0.0133	0.851	N.S.
Effect of cold	1	0.9550	0.9550	61.61	**
Interaction	4	0.0451	0.0113	0.729	N.S.
Error	137	2.13	0.0155		

Effect of temperature: t = 11.00 p < 0.001

Table VIII

		Seminal vesicles (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	0.52 ± 0.02^1 (11) ²	0.25 ± 0.01 (12)	<u>-52%</u> (23) ³
Sham-operated	(B)	0.44 ± 0.04 (11)	0.28 ± 0.02 (15)	<u>-36%</u> (26)
Partial cryptorchism	(C)	0.54 ± 0.04 (11)	0.28 ± 0.01 (10)	<u>-48%</u> (21)
Total cryptorchism	(D)	0.53 ± 0.02 (15)	0.26 ± 0.02 (23)	<u>-51%</u> (38)
Scrotal position	(E)	0.53 ± 0.02 (15)	0.29 ± 0.01 (26)	<u>-45%</u> (41)

1: Standard error of the mean.

---: $p < 0.001$

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	4	0.0504	0.0126	0.60	N.S.
Effect of temperature	1	1.9500	1.9500	92.42	**
Interaction	4	0.5600	0.0140	0.66	N.S.
Error	139	2.93	0.0211		

Effect of temperature: $t = 17.14$ $p < 0.001$

iii) Stress indicators.

Adrenal glands and thymus are known to react to cold in the direction of a stress response, adrenal hypertrophy and thymolytic regression; the surgical manipulations and the location of the testis (cryptorchid or normal), do not seem to cause any significant alteration in the gross weight of these organs (Tables IX and X).

Table IX

Adrenals (weight in milligrams)			
Groups	20°C (N)	-5°C (F)	Difference
Absolute controls (A)	52.9 ± 1.5 ¹ (11) ²	64.4 ± 3.3 (13)	+ <u>15%</u> (24) ³
Sham-operated (B)	49.7 ± 2.5 (9)	65.1 ± 2.8 (15)	+ <u>23%</u> (24)
Partial cryptorchism (C)	50.6 ± 1.7 (11)	61.8 ± 2.5 (10)	+ <u>11%</u> (21)
Total cryptorchism (D)	50.7 ± 1.3 (15)	64.1 ± 1.7 (24)	+ <u>21%</u> (39)
Scrotal position (E)	51.8 ± 2.3 (15)	61.6 ± 1.3 (25)	+ <u>16%</u> (40)

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

—: P < 0.001

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	4	79.20	19.80	0.19	N.S.
Effect of cold	1	5032.0	5032.0	47.98	**
Interaction	4	137.8	34.46	0.33	N.S.
Error:	138	14474.0	104.88		
Effect of cold:	t = 9.68	p < 0.001			

Table X

		Thymus (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	0.40 ± 0.03 ¹ (10) ²	0.14 ± 0.02 (13)	<u>-65%</u> (23) ³
Sham-operated	(B)	0.37 ± 0.01 (8)	0.15 ± 0.02 (15)	<u>-60%</u> (23)
Partial cryptorchism	(C)	0.42 ± 0.06 (10)	0.17 ± 0.03 (10)	<u>-60%</u> (20)
Total cryptorchism	(D)	0.41 ± 0.02 (15)	0.14 ± 0.01 (24)	<u>-66%</u> (39)
Scrotal position	(E)	0.37 ± 0.02 (15)	0.16 ± 0.01 (25)	<u>-57%</u> (40)

1: Standard error of the mean.

 : p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	4	0.3156	0.0789	2.92	N.S.
Effect of cold	1	1.84	1.84	68.1	**
Interaction	4	0.0550	0.0138	0.51	N.S.
Error	135	3.069	0.027		

Effect of cold: t = 15.6 p < 0.001

4. Other organs.

As a sample of other organs, the pituitary and the liver were investigated. In the case of the pituitary (Table XI), although the weight data cannot be considered reliable information, no significant alteration is observed due to cryptorchism or to cold; however, in the cold-exposed subjects, the weight values seem to crowd in the lower part of the range. The variation expressed in % of the control follows fairly closely the prediction curves of Chapter 5.

The liver (Table XII) does not yield information, except that no difference due to cryptorchism can be detected; the effect of cold noted in previous chapters, falls within the range of normal variation.

Table XI

Pituitary (weight in milligrams)				
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	11.2 ± 0.5 ¹ (12) ²	9.3 ± 0.4 (13)	-17% (25) ³
Sham-operated	(B)	10.3 ± 0.4 (9)	9.7 ± 0.3 (15)	- 6% (24)
Partial cryptorchism	(C)	11.0 ± 0.5 (11)	10.0 ± 0.4 (10)	- 9% (21)
Total cryptorchism	(D)	11.6 ± 0.4 (15)	10.2 ± 0.3 (24)	-12% (39)
Scrotal position	(E)	11.4 ± 0.4 (15)	9.9 ± 0.3 (25)	-13% (40)

- 1: Standard error of the mean. ____: < 0.01
 2: Group population.
 3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F obs.	
Effect of treatment	4	188.54	47.14	18.34	**
Effect of cold	1	56.80	56.80	22.10	**
Interaction	4	6.61	1.65	0.64	N.S.
Error:	139	357.5	2.57		
Effect of treatment:	A	B	C	D	E
Effect of cold:	t = 5.42 p < 0.001				

Table XII

		Liver (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(A)	14.9 ± 0.5 ¹ (12) ²	12.8 ± 0.5 (13)	-11% (25) ³
Sham-operated	(B)	14.2 ± 0.4 (9)	13.5 ± 0.5 (15)	- 5% (24)
Partial cryptorchism	(C)	13.1 ± 0.6 (11)	14.2 ± 0.4 (10)	+ 7% (21)
Total cryptorchism	(D)	14.4 ± 0.5 (15)	13.1 ± 0.4 (24)	- 9% (39)
Scrotal position	(E)	13.8 ± 0.6 (15)	12.9 ± 0.4 (26)	- 7% (41)

1: Standard error of the mean.

—: $p < 0.01$

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	4	4.30	1.07	0.27	N.S.
Effect of cold	1	21.56	21.56	5.46	*
Interaction	4	31.64	7.91	2.00	N.S.

Error: 140 552.7 3.95

Effect of cold: $t = 2.90$ $p < 0.01$

5. Microscopic observations.

1) Testes (Plates I and II)

a) Cryptorchid testis at room temperature (Plate I, Fig. 3; Plate II, Fig. 8). All sections display in various degrees, the distinctive features classically described by Moore (13). The tubules are disorganized and shrunk in diameter. Spermatogenesis is arrested at the Spermatocyte-I stage; few spermatocytes are seen and in many cases, the spermatogonia themselves are considerably decreased in number. Thus, few germinal elements survive and the supporting structures become the predominant visual characteristic: connective tissue and Sertoli cells.

The interstitial spaces however, do not present any degenerative modification and the Leydig elements appear entirely normal in shape, size and cellular contents (14, 15).

b) Cold-exposed testis (Plate I, Fig. 2; Plate II, Fig. 7).

The tubular diameter appears slightly smaller than normal; the disorganization is less dramatic than in the cryptorchid testis at room temperature, but considerable deterioration of the germinal epithelium may be seen (16). Spermatogenesis is depressed or even absent, although a number of tubules may still be active; the arrest of spermatogenesis, when observed seems to occur between the stages of Cyte-I and Cyte-II. Occasionally, a number of giant berry-like multinucleated cells will be seen, probably due to coalescing spermatids

(Plate I, Fig. 5; Plate II, Fig. 10).

The cells of Leydig are often small or elongated and approach the "spindle-shape" given as a sign of deterioration; many nuclei are pycnotic (3, 17); a severe mechanical disorganization of the interstitial spaces is fairly common instead of the usual triangular shape.

c) Cold-exposed cryptorchid testis (Plate I, Fig. 4; Plate II, Fig. 9). The cold-treated cryptorchid testis shows simultaneously degeneration of the tubules ascribable to cryptorchism per se and degeneration of the interstitial spaces due to cold.

2) Prostate (Plates III and IV).

a) Cryptorchism per se: by microscopic examination, it is impossible to distinguish the control prostate from the prostate of a unilateral cryptorchid and/or of a bilateral cryptorchid (Fig. 5, Fig. 11), all of which lived at room temperature.

The lobules or acini appear entirely normal in size and the epithelial cells display the normal characteristics: columnar height, position of the nucleus (basal third or fourth) near the interlobular or basal connective elements, clear space in the apical (luminal) pole of the cell, good appearance of the interacinar connective elements.

b) Cold per se: the following features are typical of the prostate in the cold-exposed animal, whether the testis is in normal position (Fig. 2, Fig. 8), or cryptorchid partially (Fig. 4, Fig. 10) or cryptorchid totally (Fig. 6, Fig. 12).

The degenerative process is unmistakably demonstrated by the height of the acinar epithelium; at best, the cells are cuboidal and, often, squamous, with the nuclei raising the cell wall and giving a picture similar to that of the capillary endothelium. Vacuoles appear occasionally in the cytoplasm and the apical clear space is consistently absent; the interacinar connective layers are exaggerated or confused. The central lobules of the cold-treated prostate show the greatest amount of degeneration and present the classical picture of the prostate from a castrated subject (3).

DISCUSSION

1. Effect of Cryptorchism.

i) On the body growth.

No somatic changes such as loss of weight, retardation of growth, can be ascribed to cryptorchism; frequent inspection of the growth curves throughout the procedure confirmed the absence of interference with the normal process of development; the terminal body weights (Table II) show no difference due to cryptorchism per se.

Thymus and adrenal glands, recognized as indicators of the intensity of the aggression and of the magnitude of the organismic response, do not manifest any alteration pointing to any type of stress response.

The location of the testis, normal or cryptorchid, does not seem to carry any significant influence on the body functions as a whole; even the surgical manipulation failed to elicit a stress reaction and

thus may be considered non-traumatic and of no consequence in the economy of the organism.

ii) On the testis.

a) Exocrine function (spermatogenic). - The data submitted represent an advanced state of testicular degeneration consequent to cryptorchism per se. Cryptorchism may, therefore, be assumed to cause changes restricted topically to the germinal sequence of the testis (14, 15). Indeed, the depression due to bilateral cryptorchism is almost exactly twice that induced by unilateral cryptorchism, (62% vs 32%) calculated on the sum of the two testes or on the right or left testis taken separately.

b) Endocrine function. Although the morphology of the interstitium appears normal, information on the functional level of the endocrine testis should be secured from the accessories.

The sex accessories, prostate and seminal vesicles, have always been considered to be true indicators of the level of the endocrine activity of the testis (3); our experience constantly showed this criterion to carry the highest order of correlation and reliability. These accessories measure only the androgenic activity of the testis and give no information on the reproductive ability. The characteristic weight level of the accessories, at normal temperature or in the cold, does not vary more than 5% due to cryptorchism per se. Hence, the normal state of the accessories remains untouched by cryptorchism, showing that the endocrine function of the testis is left intact,

within the time limit of the present investigation.

2. Effect of cold.

i) On the body growth.

The somatic effects of cold have been recognized by all investigators as a composite picture of growth arrest, loss of body weight, gradual malnutrition, eventual avitaminoses; in short, a picture of insufficient maintenance despite increased compensatory attempts.

The severe stress response attendant on cold exposure, confirmed by adrenal and thymus reaction, can therefore be ascribed to cold, exclusive of the surgical manipulation and of the physiological status resulting thereof.

ii) On the testis.

a) Exocrine function. 1. On the ground that cryptorchism is a protective reflex against ambient cold acting on the scrotal region, one might assume that the fixed scrotal position of the testes (groups EN - EF) would render the testis more vulnerable to cold; our data (Table IV, V and VI) show that the weight loss due to cold is almost identical in the "scrotal" testis and in the freely movable testis of the absolute control (AN - AF).

2. Groups BN and BF (sham-operated) appear aberrant among the rather uniform set of responses recorded; indeed, the sham-operated rats kept at room temperature (BN) have a lower than normal testis weight and those exposed to cold (BF) possess testes of much higher than anticipated weights. No explanation can be offered for the

masking of the expected cold-induced degeneration; the small population figures preclude any valid inference and these erratic results could be considered as a not too rare occurrence where the observed response goes contrary to all comparable results obtained in several previous investigations.

3. A slight differential appears between controls (group A) and group E ("scrotal position"); in view of the natural variability between small populations, this difference although significant at $p < 0.02$, may be disregarded as meaningless. Thus, a "position" effect can be ruled out.

b) Endocrine function.

The interstitial spaces reveal serious damage; the observed characteristics, though qualitative and very difficult to render satisfactorily by micrography, seem acceptable in the absence of absolute criteria by which the functional ability of the interstitial cells could be assessed; even the cell population is not considered a reliable index (17).

The sex accessories. Because of a more favorable anatomical arrangement and greater ease of dissection, the seminal vesicles offered more consistent weight data. Table VIII and the analysis of variance demonstrate the effect of cold as a specific depression of the weight of the vesicles, indicating a decrease of androgenic function due to cold per se, exclusive of any other agency, such as cryptorchism or scrotal "position" of the testis. The prostate, on the other hand, because of a more delicate responsiveness to the

titer of circulating androgens, favors biochemical and histological rather than anatomical evaluation. Indeed, the prostate presents a dramatic picture in perfect correlation with the anticipated conditions of the various experimental groups, thereby confirming its value as androgenic indicator (17). The depression of the endocrine testis due to cold per se appears specific and remarkably constant. A purely subjective remark may not be out of place: in a blind diagnosis of unmarked slides, the examiner will detect the cold-treated prostates with practically 100% accuracy, but in the animals at room temperature, an error of about 15% can be expected, because the prostate of the control may show quiescent areas where the lobules appear dormant. The central lobules give better diagnostic results, probably because the plane of section is more accurately radial, while the peripheral acini, sectioned more tangentially, may show convolutions due to superimposition of cellular strata. The relative abundance and tinctorial reaction of the acinar fluid did not seem to offer any significant information. In view of this evidence, cold per se may therefore be considered responsible for a specific alteration of the endocrine function of the testis, demonstrated by a selective depression of the androgenic indicators.

Biochemical tests give more accurate and reliable information on the functional levels of androgenic activity than organ-weight data: ^{65}Zn -uptake by the dorso-lateral prostate (18), citric acid secretion by the seminal vesicles and fructose elaboration by the coagulating glands (9).

By these methods, the effects of cryptorchism on the endocrine function of the testis have been somewhat elucidated. "High phases" (Spring and Summer) and "low phases" (Fall and Winter) of ^{65}Zn -uptake have been described; a transient decrease in uptake ability appears after one week in a mature subject rendered cryptorchid during a high phase, with a return to normal within six weeks, but during a low phase, no alteration is observed (18).

The citric acid-fructose pattern falls slightly after five days post-operatively, rises to a supra-normal level at ten to fifteen days, falls again at 28 days and by the 35th day, has returned to 90% of the normal values (9).

These tests have shown that some depression occurs in the level of androgenic activity in the days following artificial cryptorchism but a return to normal or near normal functional levels, becomes observable at 28-35 days; however, these functional variations are not detectable through histological differences and would therefore escape microscopic examination (18).

One may then conclude that over a short period of time the effect of cryptorchism is transient in nature and that the accessories reflect a normal level of androgenic activity for a long post-operative period (3).

Cryptorchism consequent to a strong cremasteric reflex evoked by cold, applied or ambient, is an important component of the male response to cold; however, the effects of cryptorchism obscured the

response in the cold, of the testicular apparatus. It was necessary to obtain the disjunction between the effect of cryptorchism and the effect of cold per se, so that their respective action could be separately evaluated; the data submitted proved the attempt reasonably successful. Cryptorchism, bilateral or unilateral, does not cause any measurable degeneration of the sexual accessories; thus cryptorchism per se would affect primarily the "exocrine" testis without interfering with the androgenic function of the endocrine testis. Somatically, no change was observed; body weight, external appearance, all organs but the testes, display no departure from the normal control.

Cold however, causes a complex group of alterations, well known to current-day investigators. The somatic response is a dramatic picture of arrest of growth, loss of body weight, gradual malnutrition with eventual hypovitaminoses, in spite of the elevation of the overall metabolism (19); in short, a picture of deficitary maintenance despite increased compensatory attempts. Within the cumulative effect of cold the boundary between somatic and endocrine areas admits overlapping and interaction (20); nevertheless, within the limits of the present investigation, one could safely state that cryptorchism induces effects of non-endocrine nature, whereas cold produces effects of definite endocrine character, not essentially related to the topical location of the testis.

Worthy of note is the recent report that the interstitial cells are considerably more resistant than the spermatogenic tubules, to

various forms of radiant energy: a transient decrease of hormone production is observed one week after X-irradiation (21) or the application of microwaves (22), although no microscopic damage is evident. The temporary depression of androgenic activity subsequent to irradiation is ascribed not to interstitial cell damage, but to a depressed ICSH output; the radiation damage to the seminiferous epithelium having perhaps been a stimulus capable of eliciting changes in ICSH production. Eventually, the time sequence of events would follow a pattern similar to the post-castration changes (21).

One last question is raised to which no answer is even attempted: why are the effect of cold and cryptorchism not summated? Indeed, the weight loss of the testis due to cryptorchism amounts to approximately 60% of the control testis weight and the weight loss due to cold amounts to only 20%; furthermore, the histological damage to the germinal sequence due to cold per se (with attendant reflex cryptorchism) is far less advanced than the damage inflicted by surgical cryptorchism, irrespective of exposure to cold. The answer can only be in the form of a hypothesis: perhaps, the cold-induced cryptorchism is not as complete, continuous and permanent as the surgical cryptorchism, so that the testis would retain a limited mobility, preventing the so-called thermal action of a fixed abdominal position; or does systemic hypothermia, though eventually fatal to the animal, limit the thermal effect of cryptorchism; or does partial ischemia due to vasoconstriction, resulting in local anoxia, prevent

the thermal damage? No answer is possible, but the question is disturbing.

SUMMARY

Cryptorchism, caused surgically, and cold exposure have been studied separately and in combination: the morphological characteristics, organ weights, and histological appearance of the testes and of the main sexual accessories have been evaluated. Physiological information derived from morphological data reveals that the effect of cold is twofold: a) somatic or non-endocrine and b) purely endocrine; the two effects are superimposed and additive, but distinct. Thus, the gonadal response of the male rat to cold, within the time limit of the present investigation, assumes the appearance of a "physiological castration".

CONCLUSIONS

a) Cold per se produces inhibition of the endocrine function of the testis, demonstrated by a regression of the sexual accessories, prostate and seminal vesicles, to a level approximating that of the castrate.

b) Cryptorchism also produced (reflexly) by cold, is not per se responsible for the inhibition of the androgenic function of the testis.

Figure 7, 1.

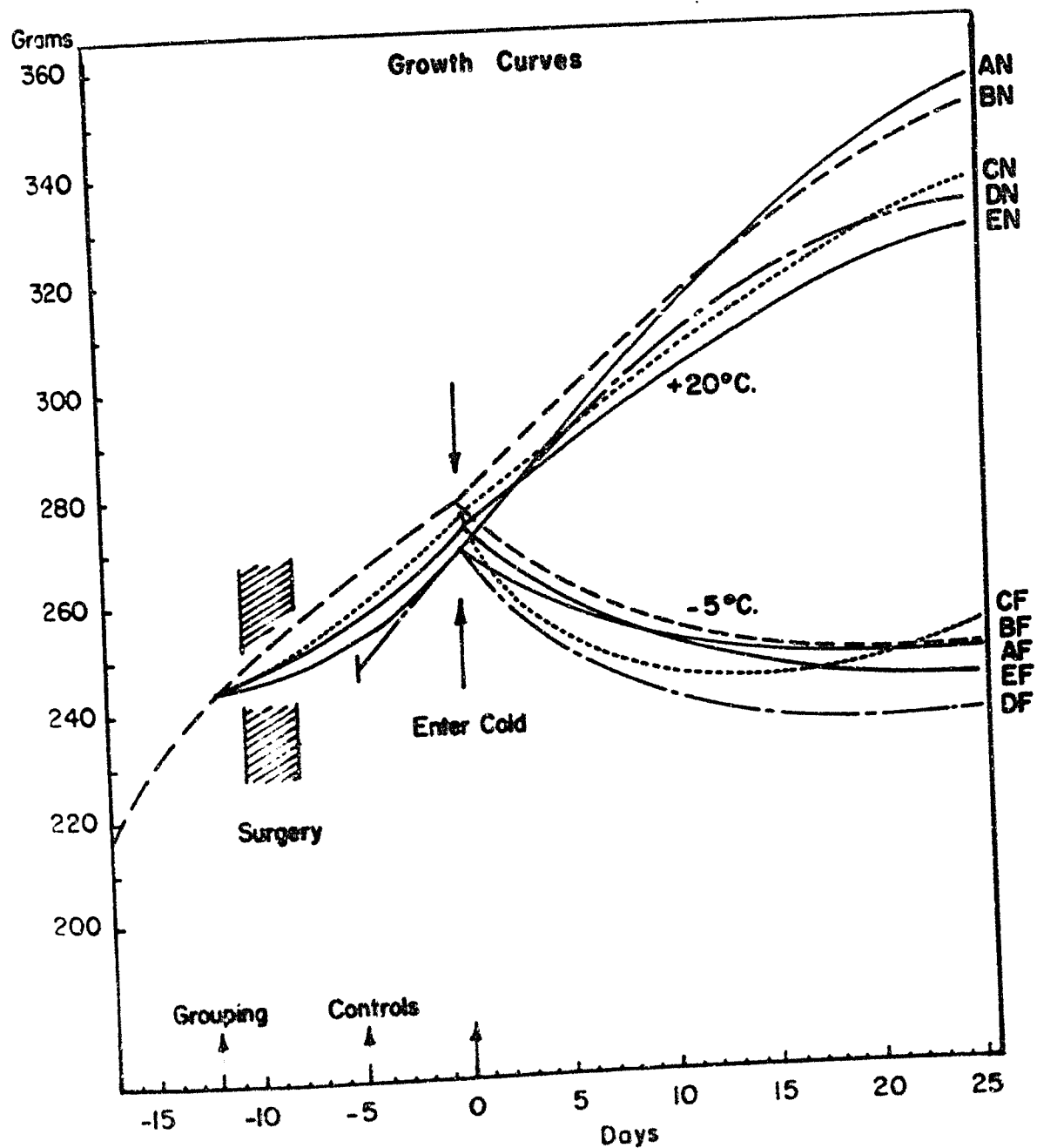


Plate I. Effect of cold and cryptorchism on the testis
of the adult rat (125 X - Mallory trichrome).

Figure 1. Control at room temperature (20°C).

Figure 2. Exposed to cold (-5°C).

Figure 3. Cryptorchid at room temperature (20°C).

Figure 4. Cryptorchid exposed to cold (-5°C).

Figure 5. Exposed to cold (-5°C) - advanced degeneration.

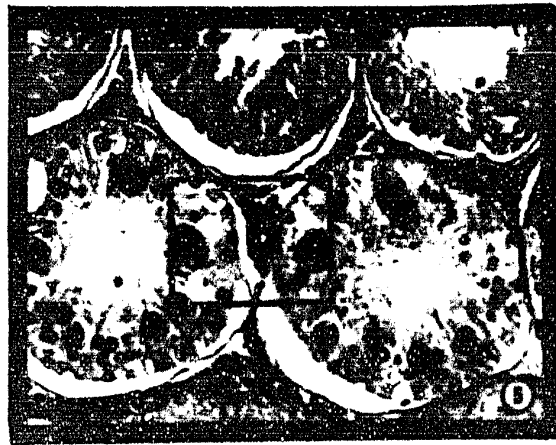
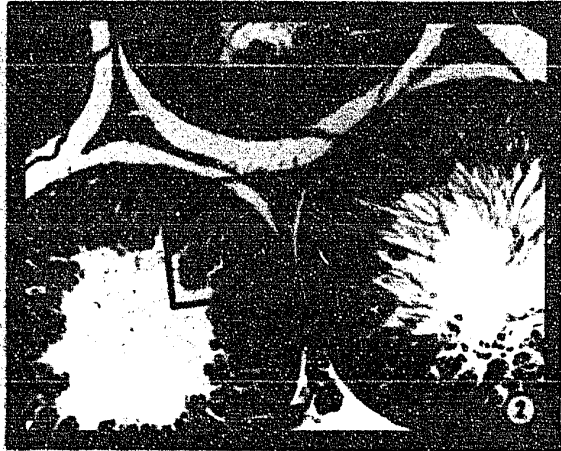
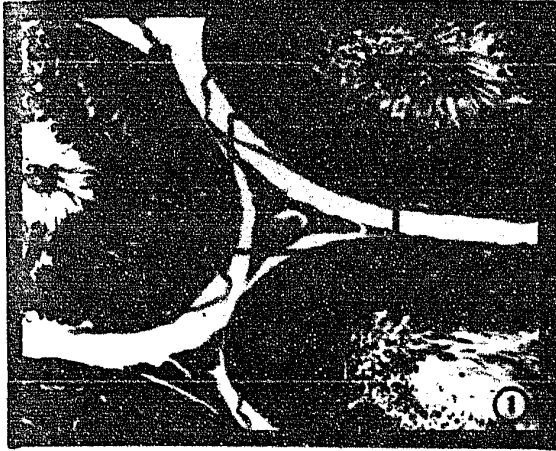


Plate II. Effect of cold and cryptorchism on the testis
of the adult rat (500 X - Mallory trichrome).

Figure 6. Control at room temperature (20°C).

Figure 7. Exposed to cold (-5°C).

Figure 8. Cryptorchid at room temperature (20°C).

Figure 9. Cryptorchid exposed to cold (-5°C).

Figure 10. Exposed to cold (-5°C) - advanced degeneration.



Plate III. Effect of cold and cryptorchism on the ventral prostate of the adult rat (125 X - Mallory trichrome).

Figure 1. Control at room temperature (20°C).

Figure 2. Exposed to cold (-5°C).

Figure 3. Partial cryptorchid at room temperature (20°C).

Figure 4. Partial cryptorchid exposed to cold (-5°C).

Figure 5. Total cryptorchid at room temperature (20°C).

Figure 6. Total cryptorchid exposed to cold (-5°C).

Plate 7, III.



Plate IV. Effect of cold and cryptorchism on the ventral prostate of the adult rat (500 X - Mallory trichrome).

Figure 7. Control at room temperature (20°C).

Figure 8. Exposed to cold (-5°C).

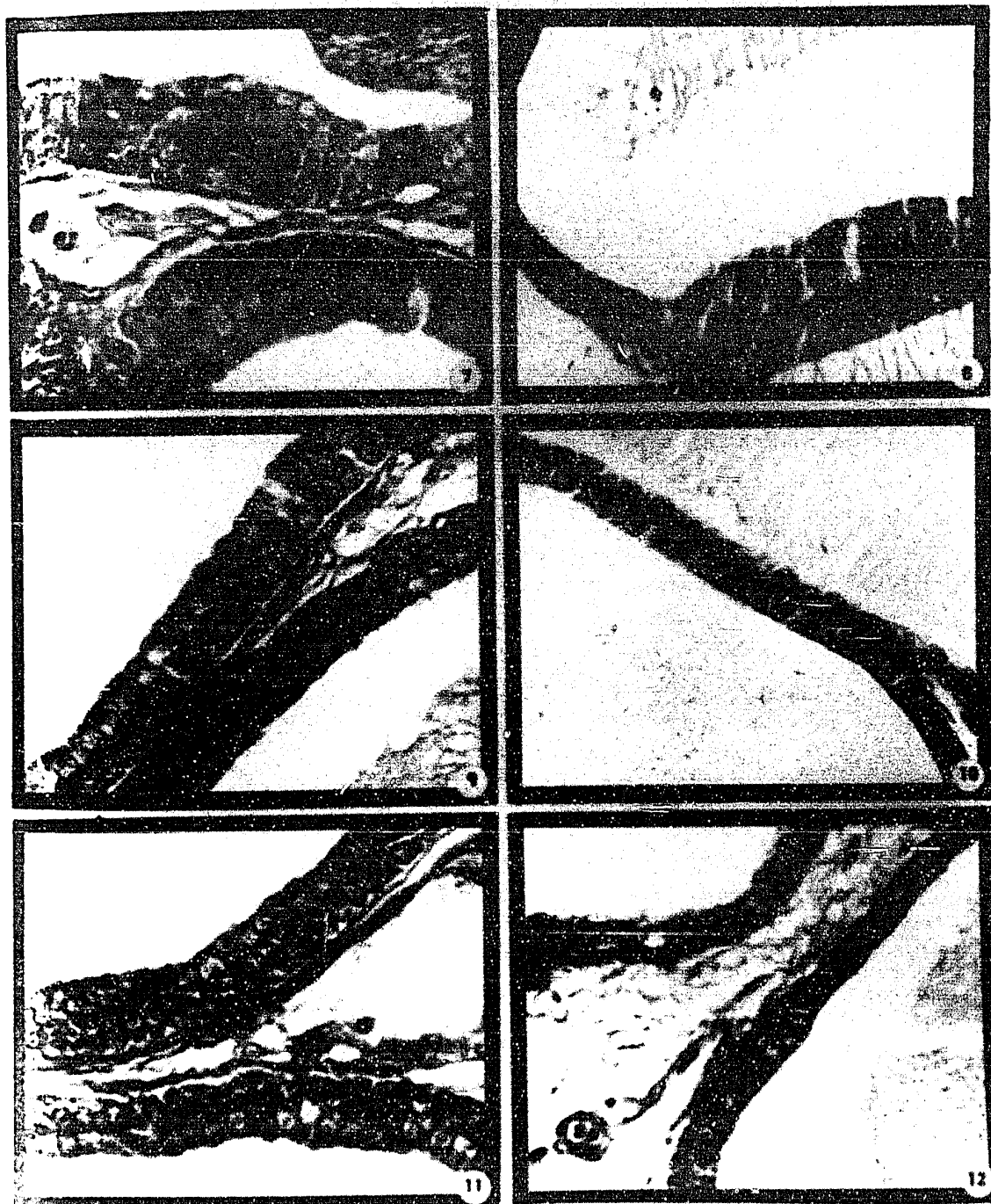
Figure 9. Partial cryptorchid at room temperature (20°C).

Figure 10. Partial cryptorchid exposed to cold (-5°C).

Figure 11. Total cryptorchid at room temperature (20°C).

Figure 12. Total cryptorchid exposed to cold (-5°C).

Plate 7, IV.



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Chapter Eight

A STUDY OF THE ANDROGENIC FUNCTION OF THE ADRENAL CORTEX

The functional relationship between the adrenal cortex and the gonad, male or female, has been the object of several investigations; the history of this research and of the conclusions obtained may be found in the substantial review papers by Parkes (1) and Courrier, Baclesse and Mardis (2). Subsequent works have examined various aspects of this complex problem: sexual dimorphism of the adrenal function (3, 4) and influence of the gonads on the cortex of various animal species (5-13).

The andromimetic action of the adrenal has especially engaged the attention of the endocrinologists; the initial observations on the growth and post-natal modifications revealed sexogenic interactions between the cortex and the male genital apparatus, in the whole animal or in the castrate under various experimental conditions (14-19). The description of the X-zone and its functional implications (20-23) led to the study of adrenal effects on sexual accessories (24) and of the peripheral tissue effects due to the adrenal androgens (25). Attempts at quantifying the andromimetic action of the adrenal cortex also led to a revision of some of the functional criteria of androgenic activity (26-28). Biochemical examination of adrenal pathologies confirmed androgen production by the cortex and permitted graded clinical evaluations (29-33). Studies on the mechanism of this relationship continue to confirm and elucidate the accepted fact of a definite action of the adrenal cortex on the reproductive function and organs (34-37).

Examination of the response of the testicular apparatus under

exposure to cold disclosed additional information on the mode of action of the adrenal cortex. Testosterone has been shown to restore adrenal integrity in hypophysectomized rats exposed to cold (38) and age, presumably accompanied by sexual maturation, enhanced cold resistance in adrenalectomized rats (39). Under a chronic (52 weeks) exposition to moderate cold (2°C), a transient atrophy of the seminal vesicles was observed, but after 40 weeks, the weight of the vesicles had returned to values above those found in controls of the same age, in spite of advanced degeneration of the testes; even the castrates, after 40 weeks at 2°C , displayed heavier vesicles (+43%) than the control castrates of the same age maintained at neutral temperature. Furthermore, a new zone in the adrenal cortex suggested a response similar in location and in appearance, to the X-zone of the mouse (40). If the rats are exposed to -5°C , a 12-day period proves sufficient to induce in the whole animal a marked degeneration of the testes and of the accessories, prostate and seminal vesicles, while the castrate under the same conditions, often displays accessories of higher weights than those of the control castrate at room temperature (41).

These two sets of observations rendered the following hypothesis particularly attractive. In the absence of the testis (complete by surgery or relative by degeneration), the adrenal cortex remains the only source of androgenic factors. This activity, normally masked by the testicular output in the normal whole animal, would be revealed in the castrate under stress, where consequent hyperactivity of the adrenal cortex would result in an increased output of cortical androgens;

on the other hand, ACTH administered to an unstressed castrate, should cause some adrenal stimulation and induce via androgen production, detectable activation of the sex accessories. The present procedure attempts a comparison, in the castrate, of the effect of ACTH with the effect of simple stress, and an examination of the response in the sexual accessories.

MATERIALS AND METHODS

Animals: 162 male rats (Wistar Albino) were arranged according to the norm of Table I.

Table I

Distribution of animals			
Description		20°C	-5°C
Absolute controls	(T)	TN (20) ¹	TF (30)
Controls + ACTH ²	(A)	AN (7)	AF (24)
Untreated castrates	(C)	CN (25)	CF (25)
Castrates + ACTH	(CA)	CAN (7)	CAF (24)

1: Group population.

2: ACTH treatment: 15 units i.p. per day, 11 days.

Surgery: 81 of these animals were castrated at 200 grams, by abdominal approach under ether anesthesia. The surgery was performed rapidly and uneventfully; recovery, swift and complete, displayed a negligible incidence of abcess or adhesions.

Maintenance: the animals were kept in air-conditioned quarters at 20-22°C, until the beginning of the exposition. After the usual 5- to 6-day quarantine, the controls (absolute) were introduced at the same weight level as the experimental animals. All subjects received ad libitum Purina Lab Chow and tap water.

Treatment: exposition to cold was made in ventilated cold rooms thermostatically maintained at $-5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and illuminated from 9:00 a.m. to 5:00 p.m. under automatic control. On day zero, when exposition to cold began, the ACTH treatment was initiated: a daily dose of 15 units was administered intraperitoneally in two injections to a total volume of 0.5 cc. The controls received the vehicle alone, Armour gelatine 40% diluted to 16% with distilled water, but under the same conditions of administration.

Data collected: body weights were obtained daily and on the same occasion, the animals were examined closely to detect the onset and evolution of cold injuries. The procedure terminated on day 12 when all animals were autopsied; after sacrifice under chloroform, the

We gratefully acknowledge receipt of ACTH (150 milligrams) from Dr. Joseph D. Fisher of the Armour Company; quoted activity: 85 units per mg (U.S.P. XV).

following organs were collected: testes, ventral prostate, seminal vesicles (emptied), adrenals, thymus, pituitary, liver and lungs. Specimens were taken for microscopy (Mallory Trichrome) of testes, prostate and adrenals.

Statistics: the raw data were subjected to the Dixon test for extremes (42); the Analysis of variance followed the "R X 2 Tables" of Yates (43), supplemented by the Duncan test (44) and/or the Student's "t". All tables quote the arithmetical mean with the standard error of the mean.

RESULTS

Body weights (Table II and Figure 1).

The significant difference noted among the "Initial Body Weights" (before exposure) results from a slight defect of synchronization between the arrival of the "Absolute" controls and the recovery of the castrates. This minor technical error seems to resorb with time and does not appear to modify the final results.

Table II

Body weights (grams)			
Groups		Preexposure	Terminal
Absolute controls	(TN)	254.7 ± 4.9 ¹ (19) ²	283.8 ± 5.1 (19)
	(TF)	249.5 ± 5.8 (14)	219.3 ± 4.4 (14) <u>-23%</u> (33) ³
Controls + ACTH	(AN)	274.1 ± 3.5 (7)	276.9 ± 6.3 (7)
	(AF)	244.1 ± 5.4 (12)	213.1 ± 3.9 (12) <u>-23%</u> (19)
Untreated castrates	(CN)	249.8 ± 4.8 (24)	288.9 ± 5.4 (24)
	(CF)	248.5 ± 4.6 (17)	218.4 ± 5.2 (17) <u>-25%</u> (41)
Castrated + ACTH	(CAN)	260.6 ± 5.1 (7)	280.3 ± 6.2 (7)
	(CAF)	250.5 ± 5.5 (16)	231.1 ± 4.0 (16) <u>-18%</u> (23)

1: Standard error of the mean.

 : p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance - summary

	Preexposure B.W.	Terminal B.W.
Effect of treatment	N.S.	N.S.
Castration	N.S.	N.S.
ACTH treatment	N.S.	t = 2.45 p < 0.05
Effect of cold	t = 2.04 p < 0.05	t = 16.3 p < 0.001
Interaction	N.S.	N.S.

At post-mortem, cold is the only factor causing a significant difference between the terminal body weights: arrest of growth and marked regression, even below the initial value. This difference is quite uniform, especially expressed in % variation from the control. Castration, as previously noted, produces no detectable effect on growth and development, at room temperature or in the cold. ACTH at room temperature, seems to delay slightly the growth of the unoperated animal and of the castrate; in the cold, the whole animal appears in unfavourable position, while the castrate seems to benefit from ACTH influence.

These remarks are confirmed by the growth curves (Figure 1) where the general trend may be seen after smoothing out of the daily fluctuations.

Survival (Table III)

Examination of the survival rates suggests the following remarks:

- 1) the ACTH-treated castrate is remarkably more resistant than all other subjects; through the period of exposure, it maintains longer a better external appearance; the signs of cold injury develop later and with a lesser morbidity;
- 2) the castrate resists better than the whole animal;
- 3) the ACTH-treated whole animal displays the lowest resistance, not much different from the "absolute" control; not only is mortality high, but the deterioration under exposure to cold is faster and more severe than among the other groups. The daily examination reveals the early onset of more serious signs of frostbite: edema of the paws and of the scrotum, necrosis of the tail and ears.

Thus, the ACTH-treated castrate survives longer and displays more effective conservation of the body weight and of the general health, while the ACTH-treated whole animal suffers diminished resistance and elevated morbidity.

Organ weights

1) Testes (Table IV): ACTH, at room temperature and in the cold, produces no significant effect on the weight of the testes; the dispersion noted does not go beyond the normal variation of body weights and remains biologically undefined. Cold causes a significant loss of weight, representing the functional regression consequent to 12 days of exposure to severe cold.

Table IV

Testes (weight in grams)				
Groups	20°C (N)	-5°C (F)	Difference	
Absolute controls (T)	3.10 ± 0.04 ¹ (19) ²	2.83 ± 0.07 (11)	- 9% (30) ³	
Controls + ACTH (A)	3.31 ± 0.04 _{+6%} (7) (26)	2.75 ± 0.06 (12)	-16% (19)	

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

—: p < 0.01

==: p < 0.001

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	1	0.053	0.053	1.48	N.S.
Effect of cold	1	6.364	6.364	178.3	**
Interaction	1	0.228	0.228	6.37	**
Error	46	1.644	0.036		
Effect of cold:	t = 6.38 p < 0.001				

2) Prostate and seminal vesicles (Tables V and VI): the effect of castration on the sexual accessories, prostate and seminal vesicles, is a classically established observation. On the 25th post-operative day, it is not surprising to observe advanced degeneration evidenced by a weight depression, to less than 10% of the control value; this degeneration conceals any possible effect of ACTH in the room-temperature castrate. The loss of weight due to ACTH in the room temperature control seems important, but it does not reach the level of statistical significance.

Table V

Prostate (weight in milligrams)				
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	308.4 ± 12.6 ¹ (19) ²	212.1 ± 15.2 (14)	- <u>31%</u> (33) ³
Controls + ACTH	(A)	248.6 ± 20.1 (7)	199.2 ± 22.3 (12)	- <u>27%</u> (19)
Untreated castrates	(C)	26.8 ± 3.0 (23)	55.8 ± 17.3 (15)	+52% (38)
Castrates + ACTH	(CA)	24.4 ± 3.0 (7)	64.9 ± 3.0 (17)	+ <u>62%</u> (24)

- 1: Standard error of the mean. : $p < 0.001$
 2: Group population.
 3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F obs.	
Effect of treatment	3	1124506.	374835.	114.8	* *
Effect of cold	1	8724.	8724.	3.37	N.S.
Interaction	3	90705.	30235.	11.68	* *
Error	106	274332.	2588.		

Effect of treatment: T A CA C

Castration $t = 17.3$ $p < 0.001$
 ACTH treatment N.S.

Table VI

Seminal vesicles (weight in milligrams)				
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	391.6 ± 18.4 ¹ (19) ²	266.4 ± 27.4 (14)	-32% (33) ³
Controls + ACTH	(A)	337.1 ± 22.8 (7)	220.8 ± 19.9 (12)	-34% (19)
Untreated castrates	(C)	48.0 ± 2.5 (24)	96.7 ± 13.9 (16)	+50% (40)
Castrates + ACTH	(CA)	49.2 ± 3.7 (7)	75.4 ± 7.7 (16)	+35% (23)

1: Standard error of the mean. —: $p < 0.01$

2: Group population.

3: Aggregate population. ==: $p < 0.001$

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	3	1603526.	534509.	144.4	**
Effect of cold	1	42167.	42167.	11.4	**
Interaction	3	180293.	61907.	16.7	**
Error	107	395960.	3701.		

Effect of treatment: T A C CA

Castration $t = 15.6$ $p < 0.001$

ACTH treatment N.S.

Effect of cold N.S.

Under the present conditions, the effect of cold is difficult to evaluate: in spite of the sophistication of the analysis of variance, the points of significant difference are buffered by the divergent direction of the effects observed. In the whole animal, the depression of the accessory weights due to cold, represents a loss of 30% by comparison to the control at room temperature. In the castrate, with or without ACTH, cold induces unexpected and significant elevation of organ weight: this increase had been considered at first as an exception, but more refined weighings revealed the remarkable constancy of the observation.

3) Adrenals (Table VII): castration in the male rat causes an increase of adrenal weight toward the values typical of the female; this increase is practically cancelled by the action of cold. ACTH stimulates the adrenal in the control and in the castrate as well, but in the whole animal, this margin decreases considerably under the action of cold. Cold per se acts in a characteristic manner only on the adrenal of the absolute control where the increase in weight is a measure of the intensity and duration of the stressing agent.

Table VII

		Adrenal glands (weight in milligrams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	45.9 ± 2.3 ¹ (18) ²	56.5 ± 1.8 (14)	+19% (32) ³
Controls + ACTH	(A)	74.5 ± 2.8 (7)	62.6 ± 3.5 (12)	-16% (19)
Untreated castrates	(C)	62.6 ± 2.3 (24)	58.6 ± 2.2 (16)	- 6% (40)
Castrates + ACTH	(CA)	77.2 ± 1.7 (7)	77.5 ± 2.7 (16)	+ 1% (23)

- 1: Standard error of the mean. —: $p < 0.01$
 2: Group population.
 3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	3	9132.8	3044.3	31.58	**
Effect of cold	1	34.9	34.9	0.36	N.S.
Interaction	3	1663.4	554.4	5.75	**
Error	106	10219.7	96.4		

Effect of treatment	CA	A	C	T
Castration	t = 4.21		p < 0.001	
ACTH treatment	t = 7.25		p < 0.001	

4) Thymus (Table VIII): thymolysis due to cold and parallel to the adrenal response to stress, displays remarkable uniformity, irrespective of the physiological status of the animal. Castration returns the thymus to juvenile levels, in the cold and at room temperature. ACTH causes the expected thymolysis irrespective of the ambient temperature, except in the cold-exposed castrates.

Table VIII

		Thymus (weight in grams)		
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	0.39 ± 0.03^1 (19) ²	0.16 ± 0.01 (14)	<u>-59%</u> (33) ³
Controls + ACTH	(A)	0.20 ± 0.03 (7)	0.07 ± 0.007 (10)	<u>-65%</u> (17)
Untreated castrates	(C)	0.62 ± 0.02 (23)	0.20 ± 0.02 (16)	<u>-68%</u> (39)
Castrates + ACTH	(CA)	0.36 ± 0.02 (7)	0.18 ± 0.02 (17)	<u>-50%</u> (24)

1: Standard error of the mean. : $p < 0.001$

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	3	0.9732	0.3244	42.13	**
Effect of cold	1	1.3664	1.3664	177.45	**
Interaction	3	0.3437	0.1149	14.92	**
Error	105	0.8091	0.0077		

Effect of treatment	<u>C</u>	<u>T</u>	CA	A
Castration				
		N.S.		
ACTH treatment		$t = 6.30$	$p < 0.001$	
Effect of cold		$t = 10.74$	$p < 0.001$	

5) Pituitary (Table IX): cold causes in all animal groups, a uniform depression predictable from the curves previously submitted, except in the ACTH-treated castrate, where this depression appears to be prevented by castration in conjunction with ACTH.

Table IX

Pituitary (weight in milligrams)				
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	10.2 ± 0.2 ¹ (19) ²	9.1 ± 0.2 (12)	<u>-11%</u> (31) ³
Controls + ACTH	(A)	10.1 ± 0.3 (7)	9.0 ± 0.4 (12)	-11% (19)
Untreated castrates	(C)	11.9 ± 0.6 (23)	10.6 ± 0.3 (16)	<u>-11%</u> (39)
Castrates + ACTH	(CA)	12.2 ± 0.8 (7)	12.3 ± 0.4 (17)	+1% (24)

1: Standard error of the mean.

2: Group population.

3: Aggregate population.

—: $p < 0.01$

==: $p < 0.001$

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F obs.	
Effect of treatment	3	110.96	36.99	12.71	**
Effect of cold	1	19.35	19.35	6.65	**
Interaction	3	6.89	2.30	0.79	N.S.
Error	105	305.2	2.90		

Effect of treatment	A	C	T	CA
Castration	t = 6.25	p < 0.001		
ACTH treatment		N.S.		
Effect of cold	t = 2.33	p < 0.05		

6) Non-endocrine organs: the weight of the liver (Table X) is rather constant: slight (significant) depression caused by cold, except in the ACTH-treated castrate. Castration favors a moderate elevation which disappears in the cold; the combination of ACTH and castration, without effect at room temperature, causes elevation in the cold, perhaps reflecting the better state of health among this group.

The lung (Table XI) presents a complex picture; cold causes in the whole animal a weight depression, emphasized by ACTH, but in the castrate cold produces an elevation of the weight value amplified by ACTH. The combination "castration-ACTH" evokes in the cold, the same relative increase already observed in the testosterone-treated castrates; no explanation is attempted of this curious effect.

Table X.

Liver (weight in grams)				
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	11.9 ± 0.3 ¹ (19) ²	11.0 ± 0.2 (14)	- 8% (33) ³
Controls + ACTH	(A)	11.9 ± 0.4 (7)	10.8 ± 0.5 (12)	- 9% (19)
Untreated castrates	(C)	13.4 ± 0.5 (24)	11.3 ± 0.2 (16)	- <u>16%</u> (40)
Castrates + ACTH	(CA)	11.4 ± 0.6 (6)	12.7 ± 0.4 (17)	+10% (23)

1: Standard error of the mean.

==: p < 0.001

2: Group population.

3: Aggregate population.

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	Fobs.	
Effect of treatment	3	22.09	7.36	2.70	N.S.
Effect of cold	1	15.06	15.06	5.53	* *
Interaction	3	35.20	11.73	4.31	* *
Error	107	291.2	2.72		

Effect of treatment N.S.

Castration t = 3.44 p < 0.02

ACTH treatment N.S.

Effect of cold t = 3.33 p < 0.05

Table XI

Lungs (weight in grams)				
Groups		20°C (N)	-5°C (F)	Difference
Absolute controls	(T)	1.53 ± 0.11^1 (19) ²	1.23 ± 0.05 (13)	-20% (32) ³
Controls + ACTH	(A)	1.52 ± 0.11 (7)	1.12 ± 0.02 (9)	-26% (16)
Untreated castrates	(C)	1.23 ± 0.03 (24)	1.39 ± 0.10 (16)	$+12\%$ (40)
Castrates + ACTH	(CA)	1.15 ± 0.03 (7)	1.54 ± 0.12 (17)	$+26\%$ (24)

- 1: Standard error of the mean. —: $p < 0.05$
 2: Group population. —: $p < 0.01$
 3: Aggregate population. ==: $p < 0.001$

Analysis of variance

Source of variance	d.f.	S.S.	M.S.	F obs.	
Effect of treatment	3	0.0900	0.0300	0.25	N.S.
Effect of cold	1	0.0352	0.0352	0.29	N.S.
Interaction	3	0.0049	0.0016	0.001	N.S.
Error	104	12.56	0.12		

HISTOLOGICAL OBSERVATIONS

Testes (Plate I).

Under the present conditions, ACTH causes no observable modification: the germinal sequence and the Leydig elements appear quite normal (Plate I, Figures 3 and 7) and without difference from the controls (Plate I, Figures 1 and 5). A biased observer would perhaps note that the number of the interstitial cells appears decreased, but not to the point where a cell count would prove imperative.

The effect of cold is marked even after this relatively short period of exposure and the damage has already reached the anatomical level. The observer will note that the animals exposed to cold, with ACTH (Plate I, Figures 4 and 8) or without ACTH (Plate I, Figures 2 and 6), show a slight elevation in the number of quiescent tubules and in some tubules, the absence of Cyte-II cells. The incidence of a certain number of pycnotic Leydig cells remains in the present case, a doubtful observation.

Prostate (Plates II and III).

The histological picture of prostate degeneration consequent to castration is a classical image (45, 46): depression of the acinar epithelium to cuboidal or even endothelial height, loss of the apical clear spaces, visual dominance of interlobular connective elements (Plate II, Figure 5; Plate III, Figure 13).

In the whole animal at room temperature (Plate II, Figure 3; Plate III, Figure 11), ACTH does not seem to cause any detectable

effect; in the cold however, activation of the prostate becomes readily observable (Plate II, Figure 4; Plate III, Figure 12), in that the epithelial cells are higher and have recovered the apical clear space. In the castrate at room temperature (Plate II, Figure 7; Plate III, Figure 15) and in the cold (Plate II, Figure 8; Plate III, Figure 16), ACTH causes a marked activation with partial reappearance of the characters of the normal cell.

The notable change in the prostate comes from the action of cold, In the whole animal, without treatment (Plate II, Figure 2; Plate III, Figure 10) or with ACTH treatment (Plate II, Figure 4; Plate III, Figure 12), the contrast is readily seen by comparison with the control at room temperature (Plate II, Figure 1; Plate III, Figure 9), in a regression of the lobules to a level almost equivalent to that of the castrate. The important and astonishing observation is provided by the untreated castrate exposed to cold (Plate II, Figure 6; Plate III, Figure 14): the acinar epithelium displays a level of regeneration comparable to that observed in the prostate of the room temperature castrate receiving ACTH (Plate II, Figure 7; Plate III, Figure 15). The absolute diameter of the lobules does not seem to possess any characteristic value; worthy of note is the fact that the observed regeneration does not reduce the amount and confusion of the interlobular connective.

DISCUSSION

In the whole animal, at room temperature, the testicular androgens reach a certain titer, resulting from all the factors whose sum constitutes the "normal state": age of the subject, state of health, functional level of the genital apparatus, season of the year, hour of the day, condition of the nutrition, etc. The adrenal androgens result from the balance "Pituitary-ACTH-Cortex" and contribute to the supply of circulating androgens. The observer may therefore read on the sexual accessories, as on a barometer, the total output of androgenic factors deriving from the sum or the synergism of the two known sources of androgens.

Exogenous ACTH given to the whole animal, causes depression of the accessories; although not significant, this depression (approximately -17% relative to the absolute control) may well result from the "braking" of the pituitary by ACTH, with an incidental reduction in the output of gonadotropins; the histology of the prostate remains normal. The slight (non-significant) elevation of testis weight among this group might be due to a liminal shift in body fluids.

Under the onslaught of aggression, especially of a measurable agency such as cold, one may observe 1) hyperactivity of the adrenal denoting the intensity and duration of the stress and 2) a characteristic depression of testicular activity. The adrenal response with thymolysis as a collateral, may well include an elevation in the production of cortical androgens, but since their titer is unknown, the massive depression of the endocrine activity of the testis becomes

the significant feature. Result: net decrease of total output of androgens, reflected in the degeneration of the sexual accessories, weight and histology.

ACTH given against this background of events, does not typically affect the weight of the accessories, but the histological modifications display a stimulation of androgenic nature, caused by the adrenal now made hyperactive by cold and by ACTH, in spite of a depressed level in the testicular output.

In the room-temperature castrate, the principal source of androgens being removed, the sexual accessories degenerate rapidly to a sub-infantile level. Might absence of the testicular braking action on the pituitary perhaps induce an overproduction of ACTH, from which would result the feminization of the adrenal of the castrate? What is then the fate of the adrenal androgens? Does the X-zone which appears in certain species under these conjunctures, display an increased activity of the sexogenic region of the cortex? In any event, the accessories degenerate progressively, proving the rapid decrease of effective androgens and/or a reduced sensitivity of the ultimate targets.

After ACTH administration, the weight of the accessories reveals a moderate weight response, but the histology shows clearly the picture of a distinct activation, the specific response of the regenerating acinar epithelium, which points definitely to an endocrinological influence by the systemic route rather than by a strictly local action.

In the cold-exposed castrate without ACTH, the activation noted in the sexual accessories can derive from one source only, the adrenal androgens. From the general response of the organism to stress, emerges an elevation of cortical production of androgens, at a level comparable to the level consequent to ACTH treatment in the room-temperature castrate. Thus, the sex accessories show repair of the weight and of the histology, partial in extent, it is true, but characteristic of the androgenic influence; ACTH superimposed to stress, does not significantly modify the level of the accessory response.

Thus, in the castrate, the stress of cold would unveil the sexogenic action of the adrenal cortex, normally concealed by the activity of the testis; it becomes therefore possible, at least theoretically, to dissociate the testicular contribution and the adrenal share to the sum total of the androgenic output deposited with the general economy of the organism. The stress, in the castrate, would manifest the androgenic action of the adrenal, minimal in extent but typical in nature. The present observations, strictly morphological in character, would require confirmation or disproving by means of a biochemical approach, where the pattern of steroidogenesis (47), utilization (48) and excretion (49) would provide a dynamic picture of androgenicity; thus, the two components would be clearly dissociated (50) and related to the broader response of the organism to the changes

in the thermal conditions of the environment (51).

CONCLUSIONS

- 1) The level of the androgenic activity of the adrenal cortex becomes observable only in the absence of the testis.
- 2) Cold in the castrate, stimulates the adrenal cortex to an increased production of androgens, the level of which becomes observable in the response of the sexual accessories.
- 3) The hyperactivity of the adrenal consequent to stress is comparable to the stimulation provided by ACTH in the non-stressed castrate.
- 4) The sexogenic influence on the accessories confirms the observation of androgenic activity of the adrenal cortex, minimal but characteristic.

Figure 8, 1.

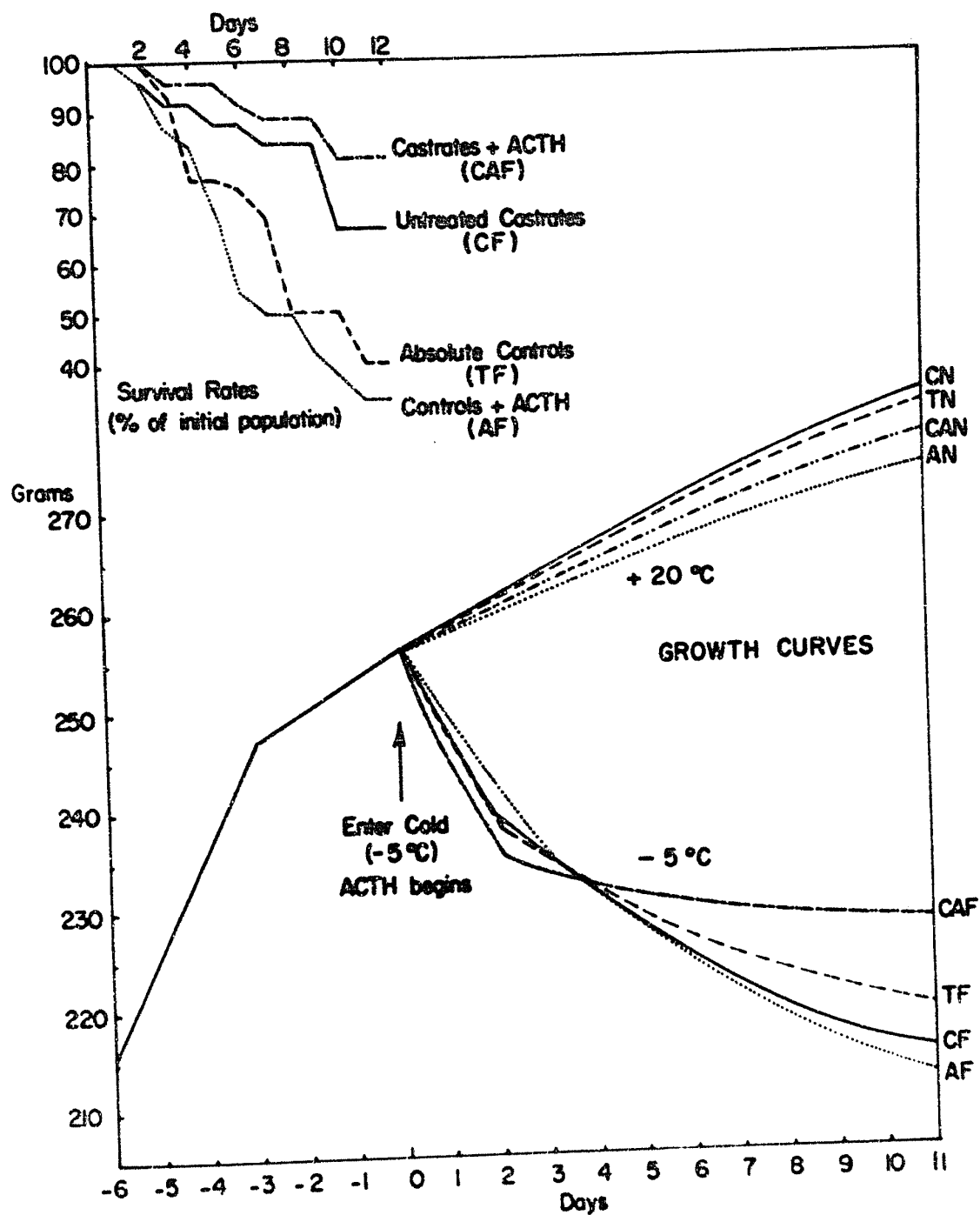


Plate I. Effect of cold and ACTH on the testis of
the adult rat (Mallory trichrome).

125 X

500 X

- Figure 1. Figure 5. Control at room temperature (20°C).
Figure 2. Figure 6. Exposed to cold (-5°C).
Figure 3. Figure 7. ACTH at room temperature (20°C).
Figure 4. Figure 8. ACTH exposed to cold (-5°C).

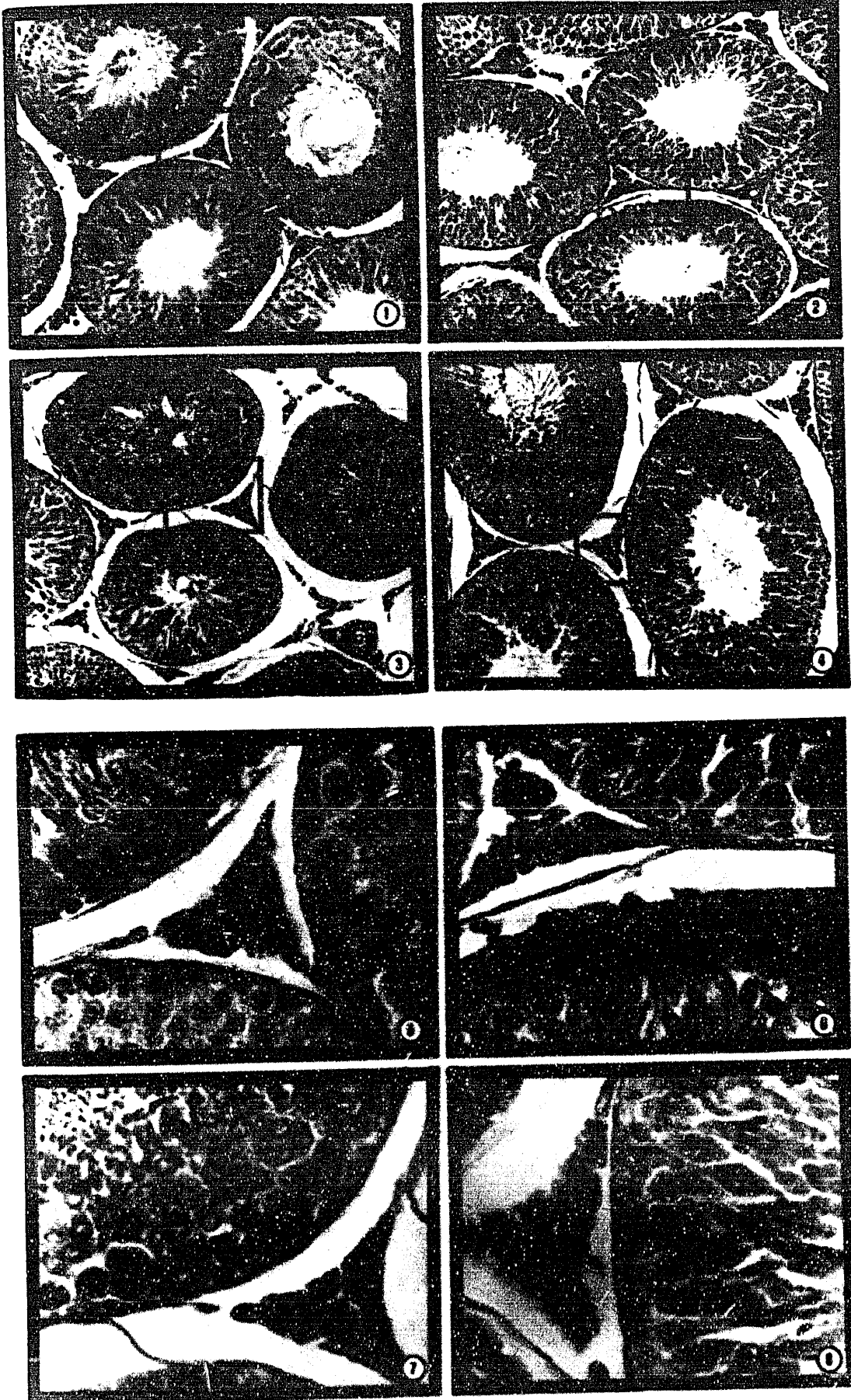


Plate II. Effect of cold, castration and ACTH on the ventral prostate of the adult rat (125 X - Mallory trichrome).

Figure 1. Control at room temperature (20°C).

Figure 2. Exposed to cold (-5°C).

Figure 3. ACTH at room temperature (20°C).

Figure 4. ACTH exposed to cold (-5°C).

Figure 5. Castrate at room temperature (20°C).

Figure 6. Castrate exposed to cold (-5°C).

Figure 7. Castrate with ACTH at room temperature (20°C).

Figure 8. Castrate with ACTH exposed to cold (-5°C).

Plate 8, II.

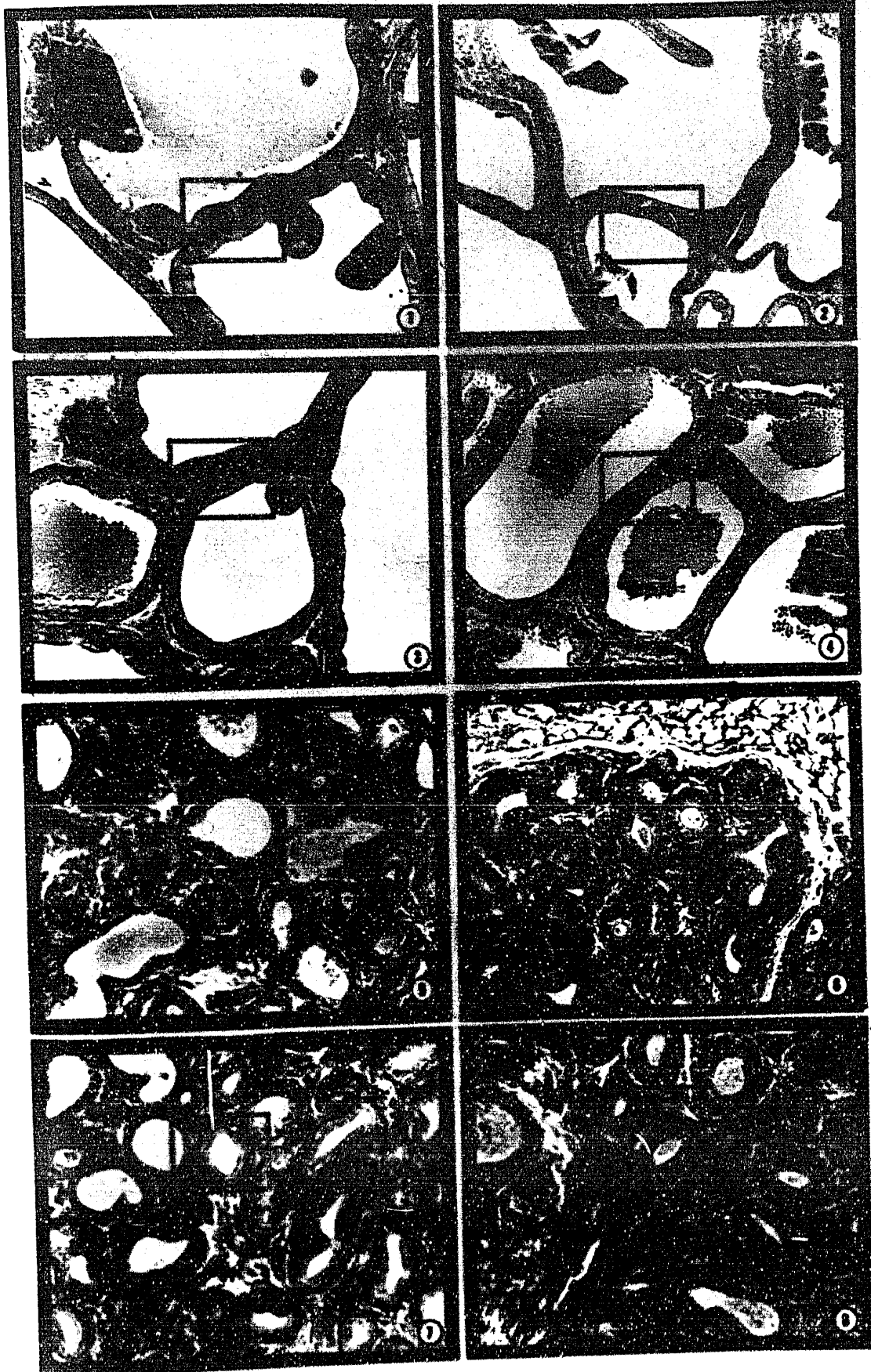


Plate III. Effect of cold, castration and ACTH on the ventral prostate of the adult rat (500 X - Mallory trichrome).

Figure 9. Control at room temperature (20°C).

Figure 10. Exposed to cold (-5°C).

Figure 11. ACTH at room temperature (20°C).

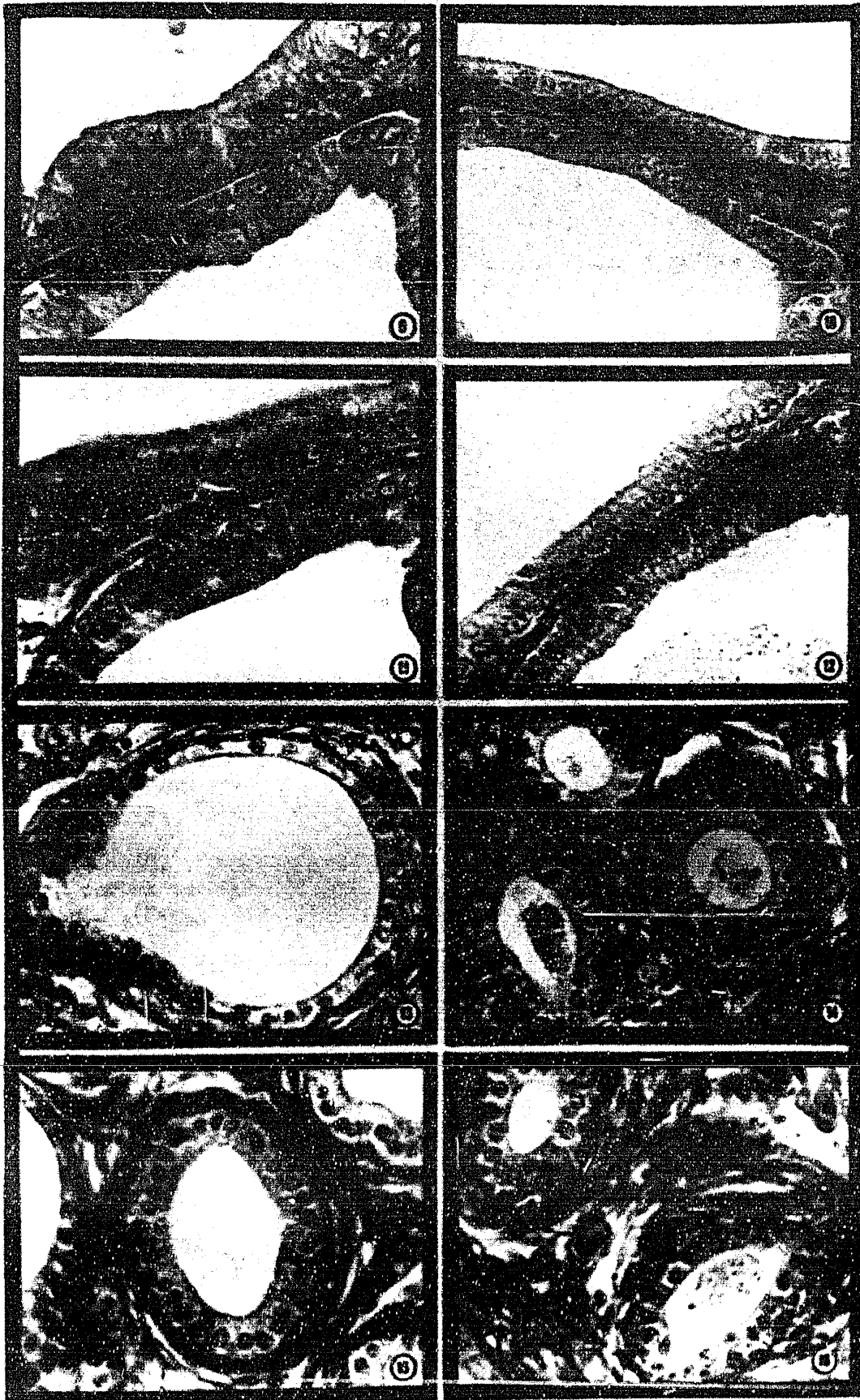
Figure 12. ACTH exposed to cold (-5°C).

Figure 13. Castrate at room temperature (20°C).

Figure 14. Castrate exposed to cold (-5°C).

Figure 15. Castrate with ACTH at room temperature (20°C).

Figure 16. Castrate with ACTH exposed to cold (-5°C).



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Chapter Nine

ON THE VALIDITY OF ORGAN WEIGHTS IN THE EVALUATION OF THE FUNCTIONAL LEVEL OF THE TESTIS

Several criteria of androgenic activity are available to the investigator, some physiological, some biochemical; some pertaining to the genital apparatus, some seemingly unrelated to this apparatus (1). Such are the changes in the submaxillary glands: increase in gland weight and of secretory granules in the apical pole of the tubular cells; they provide only a rough estimate of a qualitative response grossly reflecting the overall level of androgenicity, but they are not considered valid for the detection of a minimal androgenic action. Hypertrophy of the renal cortex in the mouse and the columnar appearance of the parietal cells of Bowman's capsule (normally squamous) have been taken to represent a qualitative and insensitive response to androgens.

The response of the sexual indicators remains the choice barometer of androgenic activity. Zn^{65} uptake, fructose and citrate concentrations, organ weight and histology, all provide a qualitative and quantitative response, firmly reliable over a wide range of androgenic stimulation; indeed, the prostate and seminal vesicle complex respond promptly and delicately and thus give a convenient, rapid and economical index of androgenic activity.

In the present investigation of the endocrine testis where detection and preliminary evaluation of potential action of cold were intended, the indices have been used with success; these criteria were preferred to the histological analysis of the Leydig cells, of uncertain value, and to the more expensive biochemical

and isotopic determinations. Several organs were also inspected for weight changes in the search of a possible relationship with androgenic disturbances. The biological value of the accepted criteria is unassailable, but a final problem remains, namely, the method of reporting changes in physical characteristics (v.g. weight of an organ) and the amenability of these data to statistical treatment.

The organization and reporting of quantitative data resulting from biological observations poses the persistent problem of the value and validity of these data; no uniform standard of reference is available and the traditional methods are fraught with uncertainty and confusion. The classical instance of this situation is the evaluation of changes in the rates of energy exchanges and their biological and clinical interpretation (2, 3, 4). The medical tradition favors expression in terms of an approximate proportionality to the surface area of the body in the species under study, with suitable corrections for application to the human species (DuBois). For the purpose of broader extrapolations in the biological field, other reference standards have been proposed, such as the "lean body mass" or the "active tissue mass". Without passing judgment on the informative value of BMR data and without choosing a position in this dilemma of scientific communication, the fact remains that all of the proposed standards appear limited in effectiveness and validity and fall under Tanner's anathema (5), where

reference standards, such as per surface area or per kilogram body weight units are "theoretically fallacious relationships based on a spurious correlation of indices" leading in some instances to the invention of new syndromes.

In actual practice, the biologist must avoid "incautious use of ratios" and unjustified extension of linear relationships into regions where they do not apply, but he may have to reconcile himself with a less effective and perhaps biased standard, provided the mode of expression is readable and reasonably reliable. The following remarks contain a recitation of the mental pathways in the selection and rationalization of a mode of expression, with undertones of self-justification. The problem could be reworded as follows; what is the best way of expressing quantitative changes (e.g. weight changes in the sex indicators and other organs) so that the differences observed (or lack of differences) reveal a true biological phenomenon and not merely a mathematical or statistical artefact; in other words, what should be the fixed point of reference or the standard of comparison?

Certain postulates must be accepted as a primary substructure.

- 1) Age can be represented by the body weight, in a certain breed of a given species; i.e. a certain physiological level (with an acceptable range of variation) represents the chronological age; thus, changes proved statistically significant, indicate a departure from the normal control and provide biologically meaningful information. Thus, hypertrophy may be taken to indicate a hyperfunctional state or

synonymously, a level of function normally associated with a more advanced age; conversely, a reduced organ weight may be taken to indicate a hypofunctional condition. Marked regression to organ weights typical of a juvenile age, will then be taken to represent a severe to extreme state of degeneration. A distinction to be detailed later, between somatic and endocrine organs will remove the apparent fallacy of this postulate.

2) Body weight and organ weight (or function) are related to each other in a certain manner, amenable to mathematical expression and scientific prediction. This relationship will not usually be linear, i.e. where the origin is zero - a case in point: the Cardiac output of a subject of zero body weight, falls beyond relevancy (5). The relationship is proportional, either of the regression type, ($Y = a + bX$) or of the power type ($Y = X^a$).

3) Changes in organ weights, especially the endocrine glands, are "trophic responses", i.e. pertaining to size of body and its parts; these changes, increase or decrease, may or may not necessarily represent respectively hyper- or hypofunction (6). The discrimination at this point is of conceptual, not mathematical order.

EXPERIMENTAL DESIGN

The experimental design may create two different situations:

1) determinations may be made at one single point in time where the object of study is the nature of the reaction in animals of uniform age (ideally, litter mates) acutely subjected to a set of

conditions; this is evidently a singular instance of the following situation.

2) determinations are plotted as a function of time in the study of the sequence of events in the development of the response over a selected period of time, during which the conditions are continuously applied; a case in point being the effect of the duration of exposure to cold (at a fixed temperature value) on the endocrine testis. In this case, the age factor of the experimental subjects cannot be neglected in the evaluation of the final results.

Modes of expression of observed changes (organ weights or other criteria) (6).

1) Absolute values. The means of the controls and of the experimentals are quoted and the difference between the means (indicating hyper- or hypotrophy) is expressed: a) as an arithmetical difference, i.e. control minus experimental; this is the "absolute difference". b) as a fraction of the control value, i.e. $\frac{\text{experimental}}{\text{control}} \times 100$; this is the "relative difference".

Remark: this method requires near-identity of the basic conditions, i.e. age and/or body weight and provides information on the nature of the response per se, where the potential age effect is removed.

Some investigators prefer to compare control and experimental on the basis of the absolute values of organ weights especially in purely physiological responses, v.g. effect of handling on rat organs (7); other authors, especially biochemists, refuse to accept a corre-

lation between body weight and organ weight (e.g. liver), and between body weight and total liver-SH groups (8), adding the caution that animals should be selected on the basis of both body weight and age.

2) Relative values. The organ weights are expressed as a fraction (%) of the body weights in the controls and in the experimental; the "fractional weights" are compared, giving a non-dimensional quotient (6). Two situations may be encountered in the experimentals:

- a) the body weight and the organ weight vary in the same direction and to the same level, leaving constant the "fractional weight" (ratio of body weight to organ weight); this is the "isometric" hypo- or hypertrophy;
- b) the body weight and the organ weight vary in different directions resulting in different fractional weights in the experimentals and in the controls; this is the "allometric" hypo- or hypertrophy.

The quotient of the two fractions (control versus experimental) gives the degree of "allometry" of the experimentals, i.e. the percentage of hypermetry (hypertrophy) or hypometry (hypotrophy).

Remark: the distinct advantage of this method resides in the fact that fractional weights give non-dimensional quotients, permitting safe and simple comparison between species where the body weights may be widely different; thus in comparative physiology, functional responses may be appraised and compared, even if an important departure from isometric build obtains between the species compared (9, 10, 11).

This approach is based 1) on the convenient (but unproven) premise that body weight and organ weight are always allometrically related, and 2) on the doubtful relationship between ingested calories (nutritional state) and organ weights. Errors of interpretation must be avoided, viz. the false conclusion that cold hypertrophies the lungs; indeed the lung weight appears identical in the controls and in the experimentals, but the body weight of the cold-exposed is significantly lower; the lungs should be considered hypermetric without hypertrophy. As a redeeming feature, the method renders explicit the change in body weight-organ weight relationship which under certain conditions, might well prove biologically significant; this mode of expression finds considerable favor and has served as a basis for a broad spectrum of studies by nutritionists, (12, 13, 14), endocrinologists (15) and various physiologists (16, 17, 18, 19).

3) Regressed values. A "regression line" defines the group variation and provides an expression of the direction of the functional change (20); comparison is thus possible between experimental groups, where the respective slopes become the characteristic and representative value. Furthermore, "extremes" are smoothed out and brought into the fold, by what amounts to a drastic curve fitting.

Remark: in one respect at least, we found that application of the Dixon test for extreme outliers resulted in a good "cleaning" of aberrant values and that the regression line did not improve the individual values by more than 2 or 3%; thus, the arithmetical means as representative of the group proved as effective as the slope of

the regression line.

The above described methods may be inserted in a system where the changes (organ weights or other criteria) are reported on a time-axis from which the time-dependency of the response can be evaluated; a standard of comparison is now needed for the estimation of the overall change where the body weight-organ weight relationship now contains a functional implication of dynamic growth.

4) Comparison against a baseline representing conditions at time zero.

The various time points are expressed as fractional (%) deviation from this initial condition which defines the origin of the time curve.

Remarks: a) the changes due to experimental conditions (hyper- or hypotrophy) are compounded with the changes due to growth, if the period of time is significantly long in relation to the life span of the animal or to a period of rapid growth. In other words, the difference between controls and experimentals includes not only the deviation due to the experimental conditions, but also the effect of age and normal growth.

b) while the organ weight among other properties relates normally to age and/or body weight in some allometric manner ($Y = aX^b$), the mode and extent of this relationship is disturbed by the experimental conditions, v.g. severe stress, so that the experimental curve of the time response becomes unpredictable.

5) Power standard or allometric relationship in the species.

Standard curves [organ weight = (Body weight)^a] defining this relationship for a given species can be found in the biological literature

and could possibly be used as a basis of comparison, i.e. representing the control conditions; the experimental response will then be expressed as a fractional (%) deviation from the control values for a similar age and/or body weight. For the rat, such curves or mathematical functions have been devised by Donaldson (21), Nelson (22) and Adolph (23).

Remarks: a) such "standard values" are not transferable from species to species (24, 25).

b) these standards appear subject to considerable variation: strain or breed differences, over a period of years and between different localities; possibly different conditions of rearing, nutrition and any number of other biologic variables, and different autopsy methods between the authors.

c) the allometric relationship is distorted during the development of the experiment and caution must be exercised in the interpretation of the results (26).

d) these data are nevertheless valuable as a guide for qualitative estimation and gross prediction, but do not provide a solid basis for quantitative evaluation of effective changes.

6). Standard curve obtained for the breed used in the course of the experiment.

We established our own Body weight-organ weight curves and the defining equations from the control animals used over an extensive course of experimentation (27); these curves served as a prediction gauge in the management of large animal populations and as a qualitative guide line in the estimation of the observed results.

Remarks: a) the changes due to experimental conditions are compounded with possible changes due to the growth process (age) in the controls and in the experimentals as well.

b) These standards are based on the unconfirmed postulate of direct and causal relationship between body weight and organ weight, where the proportionality is mathematically constant, so that to a given value of body weight, a certain value of organ weight should correspond and vice-versa. Thus a sliding scale of body weight (or age) should display organ weight in accord with the relation $Y = aX^b$ over a long fraction of the life span. Furthermore, this relationship though provisionally acceptable, applies only to the normal growth and development (controls), but since growth and development are vectorial values (with direction and sense), reversibility along the time scale is highly questionable and probably unjustified (28).

7) Control versus experimental at the same point in time.

The assumption is that the control is identical in physical age with the experimental and represents the state that the experimental should have attained, had he not been subjected to the experimental conditions; the observed changes are then expressed as fractional (%) deviations from the respective control for each time point. The final plot of the time-sequence represents the "ensemble" of these singular deviations; the curves are then fitted and the equation calculated.

Since the animals are initially chosen with a very narrow margin of body weights, they are presumed to be of practically the

same chronological age; thus, for each time point, are cancelled the effect of age or growth, the characteristics of strain or breed, as well as the influence of season and other possible cyclic variations (impossible to assess in spite of various approaches).

The observed changes are thus assumed to represent the pure response to experimental conditions, i.e. the departure between control and experimental being the aggregate response due exclusively to the imposed experimental conditions.

The normal physiological and morphological characteristics are per se vectorially associated with the chronological age rather than with the body weight; insofar as weight corresponds to age, a certain graphic relation may be expressed which is mathematically acceptable, but less conceptually satisfactory.

The organ serves as its own control and simulates conditions obtainable only with non-destructive methods, such as BMR readings over a period of time, where the value at time zero stands as the control figure.

Although the data (organ weight changes) are intrinsically poor and the curve fitting unsophisticated in the eyes of a rigorous mathematician, the information obtained proved interesting and served as the basis of an important distinction; a marked difference was thus evident between the gross responses of various organ groups, allowing discrimination of the data that could supply information on the fate of the endocrine testis under stress (cold).

Expression of functional changes

Our personal choice of a mode of expression was made as follows: in the acute experiments, absolute values (paragraph 1) of the means were analyzed for significance by means of "R X 2 tables" of Yates complemented by the Students' T test and/or the "multiple range test" of Duncan; the relative difference (paragraph 1,b) complemented by the T test, displays the fractional (%) deviation between control and experimental. In the time-schedule procedures, the "relative differences" were plotted against time, the curves fitted and defined (paragraph 7).

The curves previously submitted (chapter 5) may now be treated differently (figure 1): along the ordinate, the experimental values are expressed as a fraction of the control rather than as a fractional loss, the two "families" of curves are summarily averaged and re-plotted against time as two single curves. The "somatic" group now contains body weight and the primary endocrine organs: testes and pituitary. The weight loss represents the stress effect due to cold, an aggregate of nutritional and metabolic disturbances which may terminate in death by hypothermia. The "indicator" organs display a two-phase response: initially, the loss is rapid (half-time of 4-5 days), out of proportion with the somatic depression; this is the endocrine component of the response to cold: precipitous functional deterioration of the androgenic testis.

Later, a second and slower phase, grossly parallel to the changes in the somatic group, represents the somatic component of

accessory degeneration; this second phase seems to progress towards some asymptotic value, probably the indestructible structures of these organs.

This dynamic view, simplified and intuitive rather than mathematical, confirms the hypothesis that cold causes in the testis, a functional and morphological degeneration. As primary organs, the testis and the pituitary follow closely the vicissitudes of the overall metabolic economy; the evaluation of endocrine damage is possible only at the level of the accessories, where the somatic and endocrine effects of cold appear successively and additively (29).

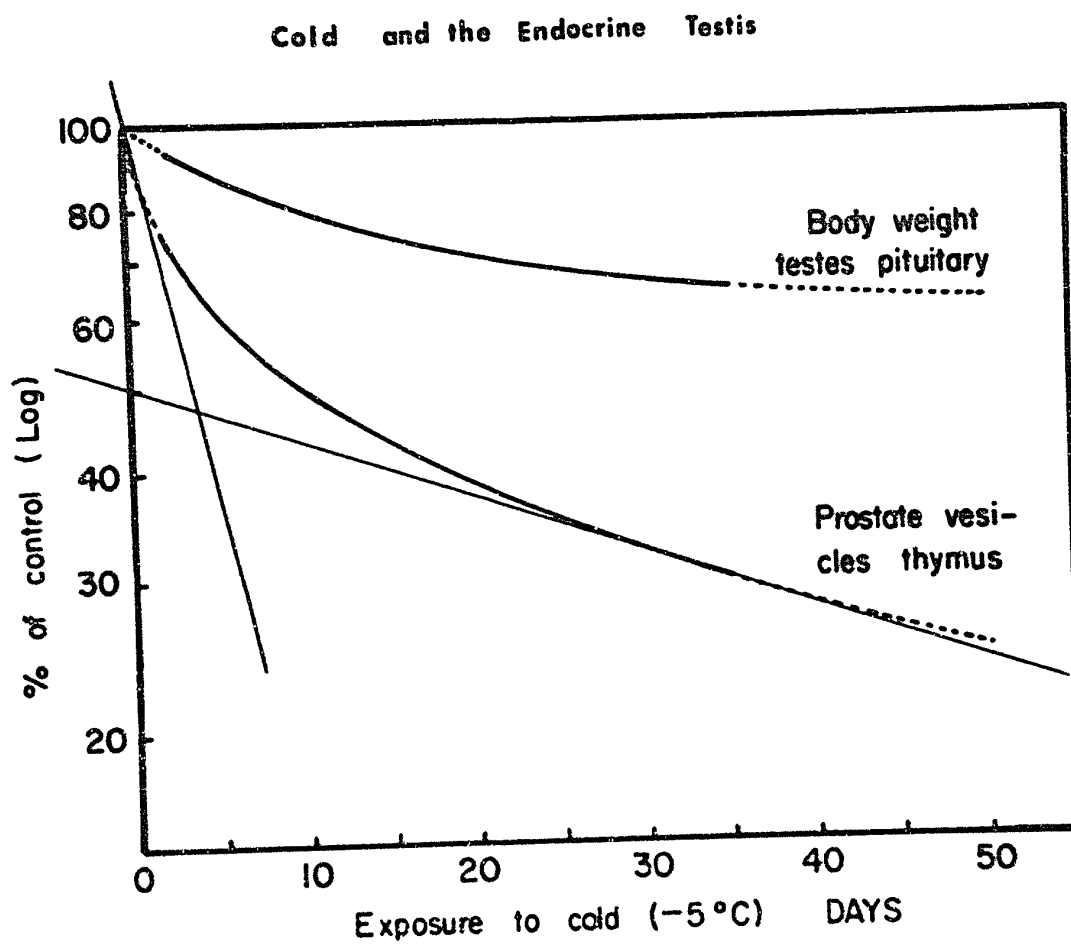
CONCLUSIONS

1. Since information on the level of androgenicity of the endocrine testis can be obtained only from the sexual accessories, the changes induced by cold in these organs must be expressed in some quantitative manner, allowing evaluation and comparison;
2. The mathematical expression was chosen after a survey of some possible forms: 1) the absolute values of control and experimental are analyzed according to the "R X 2" tables of Yates, complemented by the Student's t test and/or the Duncan F test; 2) the difference of control versus experimental is expressed as % of control and the t test is applied; 3) the time development of the phenomenon is expressed by time-curves showing the loss of weight

as a fraction of the control;

3. An intuitive rather than graphic analysis of the resulting curves confirms the hypothesis of a two-fold effect of cold on the male genital apparatus: somatic, parallel to the loss of body weight and endocrine, out of proportion with the somatic changes and representing the indicator value of the sexual accessories.

Figure 9, 1.



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SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The pituitary control over most of the endocrine glands is a long recognized fact of Endocrinology, but several modalities of this relationship remain nebulous. Some functional axes, pituitary-adrenal cortex (1-4), pituitary-gonad (5-10), receive constant attention, but interaction between the axes themselves still begs elucidation. Rules of interpretation and diagnostic signs of definitive value are still lacking in the appreciation of the functional level of the endocrines.

Recent investigations of the axis Hypothalamus-Pituitary bring to the fore new views on the integrative function of the hypothalamus with respect to the endocrine system. The physical continuity between hypothalamus and pituitary is necessary for GnRH and LH release (11). Inhibition of hypophyseal secretion results from selective hypothalamic lesions (12) and correction is obtained by suitable hypothalamic grafts (13). Implantation of testosterone propionate in the median eminence resulting in depression of spermatogenesis and prostatic involution, points to the existence of centers engaged in the negative feedback mechanism (14, 15, 16); conversely, the increased neurosecretion (observed in the interstitial latero-dorsal nuclei) following castration, is reversed by injection of testosterone derivatives (17, 18, 19).

Detailed localization studies in male and female rats with

technical refinements of considerable interest, confirm the function of the basal tuberal-median eminence area as a focus for negative feedback in the control of gonadotropin production (20). Comparative lesion studies in various animal groups observe the same gonadal degeneration, with the added note that the pituitary itself shows minimal changes and that exogenous gonadotropins exacerbate the degeneration. Because the gross gonadal regression is out of proportion with the minimal pituitary deficiency and because of correlation of lesions of the cerebral cortex in the human with genital atrophy without decrease of tropin titer, the possibility is seen of a regulation of the gonad without pituitary relay; reference is again made to the testicular atrophy brought about by bilateral lesions of the amygdaloid complex (21).

The old idea of nervous influence (somatic and vegetative) over anterior pituitary activity (and other endocrines) is thus revived, in that the neurohumoral transmission between hypothalamus and pituitary and the feedback activity of the hypothalamic nuclei are probably qualified by some form of CNS regulation (22). Thus, evolves a "concept of hypothalamic functioning", where the hypothalamus becomes the final common pathway for environmental influences on the pituitary (23). Lastly, the traditional concept of thermoregulation by the hypothalamus (24) has been re-evaluated by direct recording techniques (25) and a local hypothalamic balance of catecholamines is now considered an important factor in the maintenance of body temperature (26).

As a summary of the present venture, one would like to integrate the components of the stress response of the testicular apparatus into a clear and concise picture; the few findings reported as well as the theoretical considerations make this possibility more and more remote. Often, the findings are liminal and mark a trend rather than display a clear phenomenon, but we feel that we have validly stated a problem and opened a new and valuable avenue of research. The branching out of the initial project promises interesting results and perhaps some new insight into basic concepts and potential applications.

The present conditions are strictly experimental, in that they do not equate the natural situation of the wild environment and the dosages are selected for visible action rather than for physiological and pharmaceutical management. In this light, a speculative scheme may be unfolded where the findings are fused with hypothetical interpolations.

The mechanism of the stress response in the male genital apparatus appears not as a primary response, where a metabolic regulator adjusts to new conditions by some alteration of oxydo-reduction patterns, but as a response, secondary to several events: somatic, topical and neuro-humoral.

The somatic events concomitant with and consequent to severe cold seem to include a decreased availability of metabolites (to the organism at large and to the genital apparatus) due to the declining food intake and to the increased metabolic demands, leading to progressive anorexia and eventual emaciation. Undoubtedly, disturbances occur in the intermediary metabolism of various substances,

resulting in nutritional deficits, to wit some instances of avitaminoses and probable essential fatty acid deficiency. Distinct alterations presumably occur in the fluid-electrolyte and acid-base balances, evidenced by edema, capillary shifts, Zinc deficiency, muscular weakness possibly associated with disturbances related with adrenocortical exhaustion and to peripheral vascular impairments.

Rather little is known of the posterior pituitary responses to stress, except for some peripheral data; this important regulator of osmotic conditions and its known connections with the hypothalamic vegetative centers, certainly deserves considerable attention.

Thus, a mutual causality scheme may be envisaged, where the anabolism-catabolism imbalance causes testicular deficiency, and the androgenic depression indirectly favors catabolism. Finally, the cortico-spinal control of shivering in the skeletal musculature, brings into the picture a fasciculate and involuntary massive performance metabolically wasteful.

Among the topical factors, the local control by the nervous system (PNS or ANS) is not considered significant relative to the testis, except insofar as circulatory impairments are concerned, in that massive shunts or vasomotion may well result in genital ischemia. The abdominal temperature, known to affect severely the germinal sequence of the cryptorchid testis but to respect the androgenic ability at neutral temperature, does not result in the expected damage in the cold-exposed subject; perhaps the

central temperature is lowered enough to prevent the spermatogenic damage.

Neurohumoral events carry the maximum import; the sequence would operate as follows:

1. The hypothalamic sensors are alerted by the spino-thalamic input from the thermal receptors, the change in blood temperature, the modifications in the osmotic composition of the blood; perhaps a moderate yet significant constriction of the hypophyseal portal system also occurs. The net result is:
2. Pituitary depression, i.e. progressive functional hypopituitarism, where FSH and ICSH are momentarily reduced. Then, the incipient spermatogenic depression initiates an FSH positive feedback response, resulting in a gradually amplified depression of ICSH. At this point, the fate of the other tropins is a matter of conjecture: STH may become ineffective in sustaining conservative anabolism, TSH and ACTH, first-line emergency agents, may well increase in an inverse proportion to the ICSH and STH decrement. The net result of ICSH depression being:
3. Interstitial cell depression, where the endocrine testis approaches the eumuchoid or quasi-castrate characteristics. This biochemical injury is promptly detectable in the regression of the sex indicators; if the stress is prolonged, the anatomical damage becomes eventually observable. The androgenic depression is in the form of a deviation of steroidogenesis, by slowing or arrest of androgenesis with accumulation of the dominant precursor, cholesterol,

or by switching to an alternate pathway, towards a cholesterol or cholesterol-like termination.

4. The time-curve of androgenic depression follows a smooth decay pattern between 2 and 35 days of severe stress; additional studies not reported here, show below 2 days and above 35 days, typical phases in accord with the establishment of a true GAS sequence of events. The time-curves also show a biphasic and two-fold response, endocrine and somatic, representing the nature and the succession of events in the testicular degeneration; the overall picture is that of a functional castration, secondary to hypopituitarism induced by a non-specific stress.

COROLLARY

More problems are posed than are answered and in our minds, a number of fluid questions remain on the nature of the gonadal stress response and on the essential function of the testis.

Stress could well activate the endocrine targets and cause changes in the sensitivity threshold of peripheral tissues to various hormones, e.g. the apparent (unconfirmed) toxicity of exogenous testosterone in the stressed animal; perhaps the threshold of the hypothalamic centers is also affected, elevated or lowered. Just what is the adrenal contribution to the total circulating sex steroids under conditions of stress, where for a time at least, the cortex is considered hyperactive; is that contribution predominantly androgenic or estrogenic?

Perhaps, stress accentuates the differential processing of the gonadal hormones by the liver in the male and in the female, leading to alterations in the normal Estrogens : Androgens ratio and resulting in lowered resistance in males. Perhaps a proximity effect obtains between the testicular structures: Leydig cells, germinal sequence and Sertoli cells; perhaps the FSH : ICSH balance is disturbed, resulting in antagonism or mutual impingement on the feedback mechanisms. Perhaps the ICSH depression represents a breakdown of the homeothermal function of the hypothalamus by exhaustion, or derangement, or a "sparing" of the true emergency mechanisms, thyroid and adrenal cortex and their respective tropins, by a metabolic shunt.

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CONCLUSIONS

1. Acute exposure to severe cold causes degeneration of the testicular apparatus (morphological and physiological):
 - a) depression of spermatogenesis (exocrine) associated with cryptorchism;
 - b) reduction in the production of androgens (endocrine).
2. The degeneration caused by chronic exposure to moderate cold is duplicated in nature and compressed in time, if cold is made severe. This degeneration appears proportional to intensity and duration of the cold stress, but unrelated to age, at least in the sexually mature subject; the time-development of this damage may be visualized as a progressive sequence of exocrine and endocrine changes.
3. Ascorbic acid and Zinc, at presumed protective doses, failed to counteract the cold-induced degeneration in the testicular apparatus.
4. Adaptation to moderate cold, prior to exposure to severe cold, seems to reduce the spermatogenic damage, but the level of androgenic depression appears directly proportional to the characteristics of the severe stress, irrespective of prior adaptation.
5. Among the effects of cold, distinct areas become observable, all of which summate in the testicular apparatus:
 - a) somatic, e.g. depression of body weight and eventual emaciation;
 - b) neural, e.g. reflex cryptorchism;
 - c) endocrine, e.g. depression of androgenic production.

6. Cold per se shows no significant topical action on the sexual accessories; these organs retain their indicator value relative to the titer of circulating androgens and reflect directly the level of testosterone therapy.

7. The nature of the androgenic disturbance may be seen as a blockage of androgenesis or as a shift in steroidogenesis; no choice is made, since the moderate, (and perhaps debatable) elevation of testicular cholesterol favors both hypotheses.

8. Cryptorchism and cold act in the same direction, but independently and disjunctively; their effects do not seem to summate completely in the cold-exposed testis.

9. Adrenal androgens are unmasked in the cold-exposed castrate and seem to act minimally but typically; ACTH renders the castrate more resistant to cold, but lowers the survival of the intact animal.

10. The weight depression of an endocrine organ, e.g. testis, is not a reliable criterion of functional depression, as the loss is in the same order as the somatic depression simulated by diet restriction; within a limited range of age, no correlation seems to exist between body weight and testis weight.

11. The weight depression of secondary endocrine organs, e.g. sex accessories and thymus, is a valid indicator of functional depression in the primary organ and represents the sum total of two components:

- a) somatic, related to metabolic alterations engaging the whole organism;
- b) endocrine, consequent to changes in the functional level of the primary organ and beyond the magnitude of somatic changes.

12. Variance between absolute values rather than co-variance, was evaluated for significant differences; the "state" of endocrine organs (primary, e.g. pituitary; intermediate, e.g. testis; secondary, e.g. prostate) appears to be the resultant of a closed-circuit chemical homeostasis, upon which somatic factors exercise limited action.

13. An intuitive rather than graphic analysis confirms the biphasic and two-fold effect of cold on the male genital apparatus.

14. Cold per se acts on the testis:

- a) by causing reflex cryptorchism and its long-term effects on spermatogenesis;
- b) by way of somatic conditions resulting from metabolic deficits and eventual emaciation;
- c) by way of some neuro-humoral pathway (hypothalamico-hypophyseal) resulting in a situation comparable to castration.